Polymer composites for restoration of endodontically treated teeth

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Polymer composites for restoration of endodontically treated teeth

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Abstract

The failure of endodontically treated teeth (ETT) restored with adhesive post restorations is related to several factors but mainly associated with the mechanical and biological properties of the materials currently available to fabricate post-core systems. This study is based on developing new post materials, using a biomimetic concept to improve the survival rate of severely damaged ETT. Furthermore, the study was hypothesised that the inclusion of a polymerisable antibacterial monomer, eugenyl methacrylate (EgMA), within the resin composite core and adhesive materials would inhibit endodontic biofilm formation and lower the risk of reinfection and secondary caries.

Two experimental post materials were fabricated and optimised based on their physical and mechanical properties: (i) polyethylene-hydroxyapatite composite exhibited good aesthetics, limited water sorption and a melting temperature of 135-136 °C that would enable easy retrieval from the root canal in case of a retreatment, but their lower flexural modulus is expected to limit their use as an endodontic post and (ii) Kevlar fibre reinforced semi-IPN matrix composites showed favourable aesthetic, mechanical strength, fatigue resistance, radiopacity and cytocompatibility to function as new fibre reinforced composite post.

EgMA monomer was incorporated at 5 and 10 wt.% to formulate dual-cure composite core, and at 20 wt.% to modify two commercial dental adhesives. The antibacterial activity of these formulations was evaluated and the influence of this monomer on curing kinetics, physical and mechanical properties, wettability, bonding ability and cytotoxicity is reported. The monomer endowed the modified adhesives with obvious cavity disinfecting effects before curing and impart an effective bacteriostatic activity against oral bacteria (*S. mutans, E. faecalis and P. acnes*) commonly associated with endodontic and restorative failures after being cured, without influencing the degree of monomer conversion, bonding ability to root canal dentine and cytocompatibility.

The most suited materials developed in this study are feasible as post-core systems for effective treatment of ETT, and thus influence the practice of endodontics.

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List of abbreviations

DMA: Dynamic mechanical analysis

DSC: Differential scanning calorimetry

EgMA: Eugenyl methacrylate

ETT: Endodontically treated teeth

FTIR: Fourier transform infrared spectroscopy

GPa: Giga pascal

GFP: Glass fibre posts

HA: Hydroxyapatite

HEMA: 2-hydroxyethyl methacrylate

IC_{50}: The half maximal inhibitory concentration

IPN: Interpenetrating network

KF: Kevlar fibre

K FRC: Kevlar fibre reinforce composite

LDPE: Low-density polyethylene

MDPB: Methacryloyloxydodecyl pyridinium bromide

MDP: 10-methacryloyloxydecyl dihydrogen-phosphate

META: 4-methacryloxyethyl trimellitate anhydride

MMP: Matrix metalloproteinase

MPa: Mega pascal

MTT: Methyl thiazol tetrazolium

Pa: Pascal

PE: Polyethylene

PEM-F: Penta-methacryloxy-ethyl-cyclo-phosphazene-mono-fluoride

Phenyl-P: Methacryloxyethyl hydrogen phenyl phosphate

PTFE: Polytetrafluoroethylene

RCT: Root canal treatment

SBF: Simulated body fluid

SD: Standard deviation

SEM: Scanning electron microscopy

TiO_2: Titanium oxide

wt.%: Percentage by weight

ZrO_2: Zirconium oxide

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Introduction

Endodontic infections are caused by bacteria and treatment is directed towards removal followed by restoration (de Paz and Dahlen 2016). Apart from careful and efficient removal of bacteria, an understanding of the available restorative materials and their limitations are critical in treatment planning of endodontically treated teeth (ETT). The loss of extensive coronal tooth structure as a result of caries, trauma, endodontic therapy, and restorative procedures can often significantly weaken a pulpless tooth (Shahrbaf *et al.* 2007), posing a challenge for clinicians. A post-core restoration is generally required when the amount of remaining tooth structure is not sufficient to promote the retention of the final restoration (Mannocci and Cowie 2014). Currently, the use of fibre reinforced composite (FRC) posts in combination with dental adhesives and resin composites have widely replaced metallic posts and amalgam core materials. However, recent longitudinal clinical studies on fibre post restorations report a relatively high failure rate (Naumann *et al.* 2012; Parisi *et al.* 2015) attributed to a host of reasons such as adhesive failure, root and post fracture, recurrence of endodontic lesions, and secondary caries (Barfeie *et al.* 2015). These failures could be related to the components from which the post-core system is made, mismatch in mechanical properties with surrounding tissue, and improper fabrication or restorative technique.

This thesis aims to develop and characterise polymer composites tailored to function as post-endodontic restorative materials, utilising alternative components and techniques, with desirable properties and long-term performance of ETT. The work was initiated by providing an overview of the literature including the current principles, materials, and techniques for restoration of the endodontically treated teeth, highlighting their shortcomings based on the best evidence available (Chapter 1). The experimental studies are divided into two main sections (Figure i-1): The first section is dedicated to the studies in Chapters 2 and 3, related to exploring the development of particle reinforced composites with thermoplastic matrix and fibre reinforced composites using Kevlar in a semi-interpenetrating matrix as intracanal post materials.

The second section of this thesis describes the formulation, preparation, and characterisation of intrinsically antibacterial resin composite core material based on eugenyl methacrylate (EgMA) antibacterial monomer, which is presented in two complementary studies in Chapter 4. Chapter 5 discusses the feasibility of incorporating EgMA into two commercial dental adhesive systems for their application in post cementation to impart the antibacterial activity. In Chapter 6, the overall findings of the studies are discussed and concluded with suggestions for future work. This thesis is presented as a series of studies which were conducted to achieve the research aims, and are either published or submitted for publication in peer-reviewed journals. Their manuscripts have been reformatted to provide consistency of style and supplementary information at the end of each corresponding chapter. The references are listed at the end of the thesis.

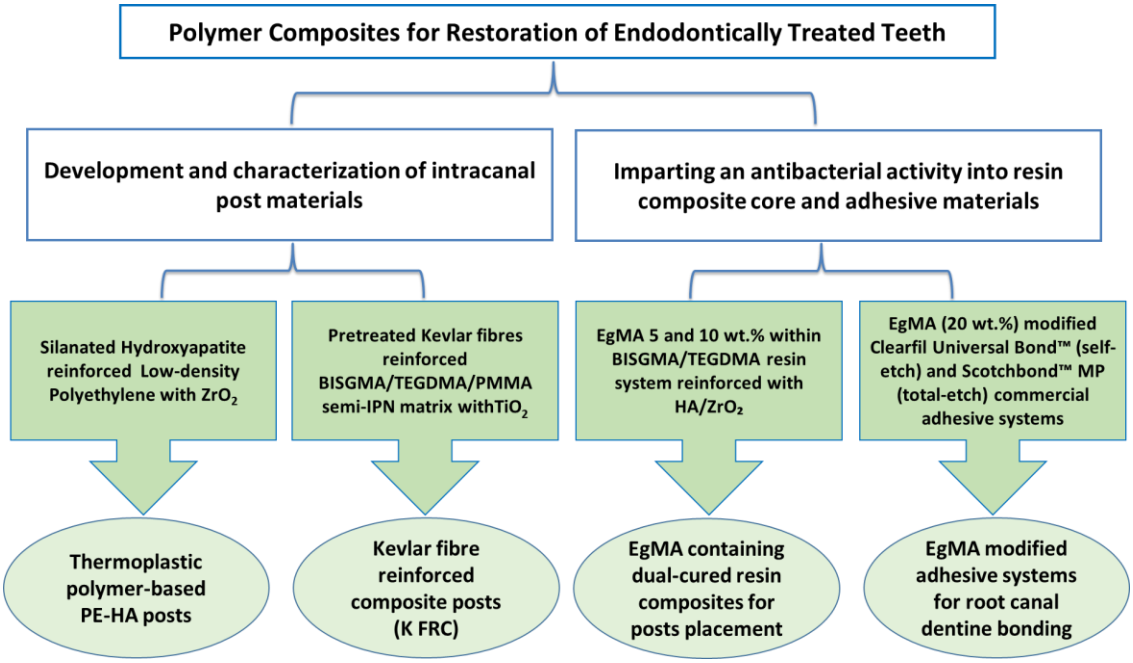

Figure i- 1 Organisational flowchart representing composites and adhesive materials developed in this study for the restoration of ETT

Chapter 1 literature review

1.1 Tooth structure

Understanding the structure and mechanical properties of teeth is important for the development of restorative materials that mimic their properties and function, as well as for bonding of different dental materials to the tooth substrate (Sakaguchi and Powers 2012). The tooth consists of three specialised calcified tissues; dentine forms the main bulk of the tooth, surrounding the neurovascular bundle in the pulp space and covered by enamel coronally and cementum on the surface of the root.

Enamel is a highly calcified tissue containing about 90 vol.% hydroxyapatite, whilst dentine contains a substantial proportion of water and organic material, primarily type I collagen (Fig. 1-1).

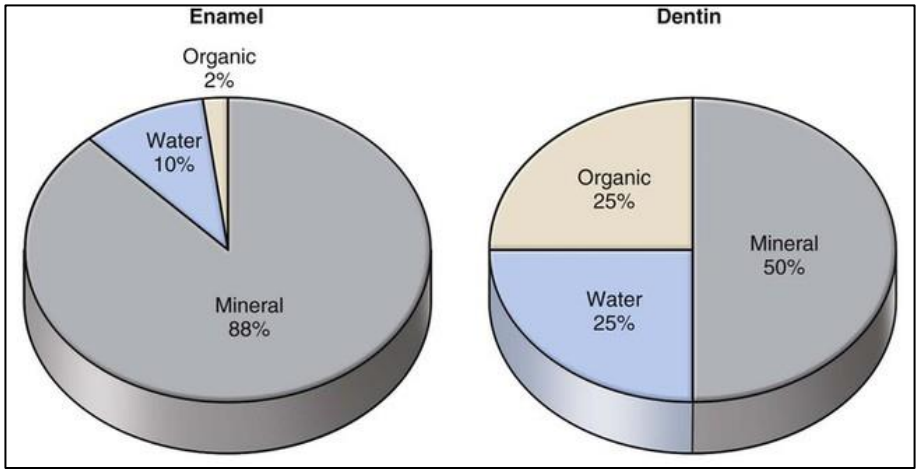

Figure 1-1 Composition of enamel and dentine by volume percent [adapted from (Heymann *et al*. 2013)]

Some important physical and mechanical properties of human enamel and dentine are presented in Table 1-1. It is important to mention that the spread of values reported in literature reflects the difficulties in performing mechanical tests on small enamel and dentine specimens. In addition, properties generally vary with direction and location of the test specimen obtained from the tooth tissue. Storage and time elapsed since extraction are also important considerations.

Table 1-1 Properties of human enamel and dentine [adapted from (Sakaguchi and Powers 2012)]

Property	Enamel	Dentine
Density (g/cm^3)	2.96	2.1
Elastic Modulus (GPa)	84.0	17
Ultimate tensile strength (MPa)	10.0	98
Compressive strength (MPa)	384	297
Flexural strength (MPa)	60-90	245-280
Shear strength (MPa)	90	138
Microhardness (KHN)	343	68
Radiopacity (mm Al)	2	1

1.2 Dental caries and pulpal disease

Dental caries (tooth decay) is one of the most widely spread chronic diseases. It is initiated by the action of bacteria within the oral biofilm which ferments the dietary carbohydrate on tooth surfaces and produces organic acids as by-products. This creates the cariogenic environment that causes demineralisation of the enamel surface and further destruction of tooth structures (Ghezzi 2014). As the bacteria penetrate towards the pulp, an inflammation, known as pulpitis is initiated and can lead to the pulp death (necrosis), where the tooth becomes non-vital. If the necrotic pulp tissues left inside the root canal space, it can undergo autolysis and releases breakdown products into the surrounding tissues resulting in periapical irritation or abscess formation (apical periodontitis) (Kidd 1999) and root canal treatment or tooth extraction are ultimately required.

1.3 Endodontic therapy

Endodontic therapy is indicated to remove the irreversibly inflamed or infected pulp from the root canal space by chemo-mechanical debridement; followed by obturation of the shaped root canal system with an appropriate material (usually gutta-percha and sealer) to prevent its reinfection with the aim of retaining the tooth function within the dental arch (Torabinejad *et al.* 2003). This set of procedures is commonly referred to as a root canal

treatment (RCT). The overwhelming success of RCT allows the retention of most damaged teeth, which were previously consigned for extraction. The clinical and radiographic signs of periapical healing are the main prognostic factor determining the outcome of primary RCT. However, other researchers have considered tooth survival as an outcome measure (Ng *et al.* 2010; Pirani *et al.* 2015). When endodontic therapy is performed under controlled clinical conditions, the overall success rates of non-surgical RCT can range between 85% to 90% (Imura *et al.* 2007) and a survival rate of 87.1% after 10 years (Pirani *et al.* 2015).

1.4 The effect of endodontic therapy

Endodontically treated teeth (ETT) are more susceptible to fracture than vital teeth, which has been related to the physical changes in the dentine of pulpless teeth including the reduction in moisture (Helfer *et al.* 1972) and loss of collagen cross-linking (Rivera and Yamauchi 1993). However, the current consensus is that the reduction of tooth structure following endodontic and restorative treatments is the main cause of fracture in ETT (Bitter *et al.* 2009a; Ibrahim *et al.* 2016). The loss of structural integrity associated with the access preparation leads to more cuspal deflection during function and increases the risk of tooth fracture and microleakage at the margins of the restorations (Pereira *et al.* 2013).

Pulpless teeth lack protective feedback mechanism (proprioception) that may protect teeth from fractures; however, the tactile sensitivity has been recently reported to be similar in both vital and non-vital teeth (Schneider *et al.* 2014). Procedures such as instrumentation, irrigation, medication, and obturation during RCT are factors that also affect ETT in terms of further loss of tooth structure, dehydration of dentine and collagen structure alteration (Mannocci *et al.* 2008; Tang *et al.* 2010). As a result of these cumulative effects, ETT become weaker and require special considerations for the final restoration.

1.5 Restoration of ETT and principles for post placement

Following the completion of RCT, the tooth must be restored to optimal form, function, and aesthetics. Endodontic treatment should not be considered complete until the coronal restoration has been placed to prevent any coronal leakage that may lead to bacterial contamination of the root canal space and subsequent development of apical periodontitis (Sagsen and Aslan 2006). The recommendation is that immediate restoration of a root filled tooth is carried out whenever possible (Heling *et al.* 2002).

The preservation of tooth structure should be considered as a primary goal, both during root canal treatment and subsequent coronal restorations. An excessive removal of dentine can change the flexural behaviour and resistance to fracture (Bateman *et al.* 2003). Furthermore, conserving at least one remaining coronal wall (Cagidiaco *et al.* 2008a) or a circumferential dentine collar of 1.5 to 2 mm ferrule effect (Fig. 1-2) has been shown to be significantly associated with a reduction in the risk of failure (Mancebo *et al.* 2010). However, other baseline factors including the restorative procedures and material selection are also essential components, influencing both treatment outcome (Schmitter and Hamadi 2011) and tooth survival (De Backer *et al.* 2007).

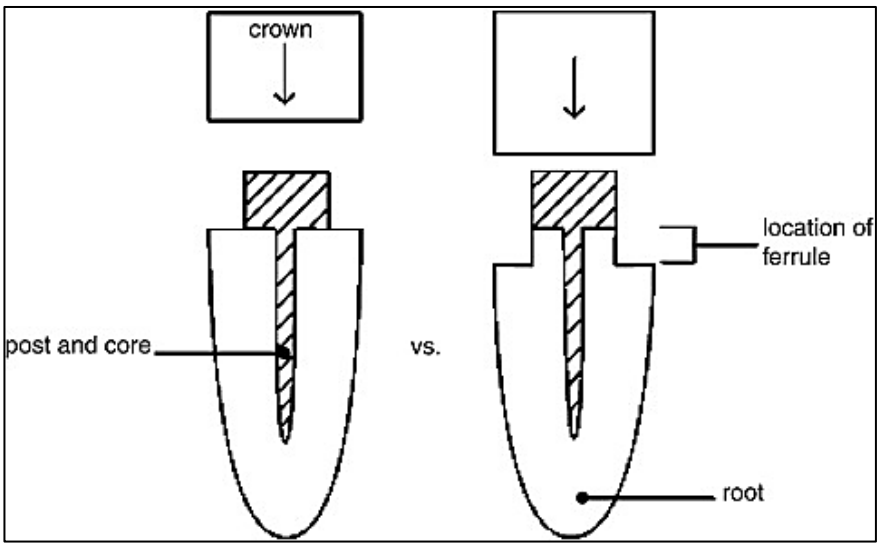

Figure 1-2 A schematic of endodontically treated tooth restored with post and core system and a crown; the coronal extension of dentine above the shoulder provides an effective ferrule [adapted from (Stankiewicz and Wilson 2002)]

Owing to the complexity of treatment procedures and the wide range of restorative materials, techniques and systems available, the restoration of ETT frequently presents a challenge for the clinician (Skupien *et al.* 2013) especially when an extensive amount of tooth structure is lost.

Post restoration is generally indicated if the amount of residual tooth structure is not sufficient to support or retain a composite or amalgam core (Mannocci and Cowie 2014). The post (dowel) also play a role in stress distribution, the use of restorative materials with biomechanical characteristics similar to dentine allows for relatively uniform stress distribution on the tooth and surrounding tissue protecting the root from the fracture (Fig. 1-3).

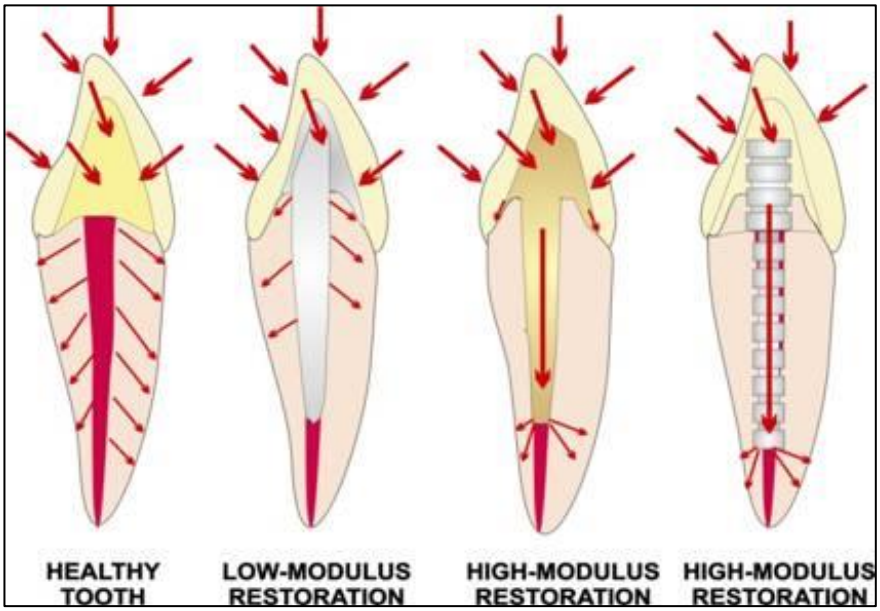

Figure 1-3 A schematic illustrating the function of intracanal post restoration stiffness on stresses distribution throughout the root of ETT. Materials (left to right), Gutta-percha, fibre post, cast metal post and prefabricated metal post. [adapted from http://www.rtd-dental.eu /GB/why_post.php]

The concept that post placement does not reinforce a tooth remains debatable (Gluskin *et al.* 2014). To date, both clinical and laboratory data provide no evidence that posts can actually strengthen the roots (Dietschi *et al.* 2008; Liddelow and Carmichael 2016). A long-term clinical study indicated that endodontically treated teeth restored with fibre post–retained restorations yielded statistically significantly higher survival and success

rates with lower incidence of root fracture compared with those of teeth without a post (Guldener *et al.* 2017).

A recent systematic review and some randomised clinical trial studies also reported that the adhesive placement of less rigid posts (FRC posts) with mechanical properties closer to those of dentine can enhance the fracture resistance of ETT and significantly reduce the catastrophic failure risk compared to stiffer materials, such as metal posts (Schmitter and Hamadi 2011; Zhu *et al.* 2015). In contrast, a controlled clinical study of up to 17 years (Fokkinga *et al.* 2008) and another 5 to 10 years (Jung *et al.* 2007), reported that teeth restored without posts performed similarly to those restored with prefabricated metal and cast posts.

Since a post may not "strengthen" or "reinforce" an ETT and the preparation of a post space may increase the risk of root fracture and treatment failure (Pilo *et al.* 2008), the decision whether to use a post in any clinical situation must be made carefully whilst taking into consideration the remaining sound coronal tooth structure, anatomic position of the tooth, functional demand and aesthetic requirement (Jotkowitz and Samet 2010).

For anterior teeth with minimal loss of tooth structure, endodontic posts should be avoided and a bonded composite resin is appropriate to seal the access cavity (Cheung 2005). However, if an endodontically treated anterior tooth is to receive a crown, a post is often indicated to provide adequate retention and resistance against lateral and shearing forces.

Molars in most cases do not require posts placement, the pulp chamber may provide an adequate retention for a core build-up and they usually receive a cuspal coverage restoration after endodontic treatment to resist the primarily vertical forces received. On the other hand, premolars, which are more likely to be subjected to lateral forces during mastication, have less tooth structure left after RCT with a smaller pulp chamber to provide adequate resistance and retention of a crown, therefore post placement is more often needed than molars (Schwartz and Robbins 2004).

1.6 Dental post systems

The concept of using the root of a tooth to provide the retention of a crown was introduced in Tokugawa era (1603 to 1867) when the Japanese used a wooden dental restoration designed to function as a root canal dowel (Ring 1992). This concept formed the basis of the modern post and core restorations.

Dental posts may be either individually casted together with the core, or prefabricated. The endodontic post restoration involves removal of gutta-percha from the root canal to prepare a space into which the post is fitted and cemented, leaving several millimetres protruding to retain a core that supports the final restoration (Fig. 1-4). Cement with or without bonding agent is usually used to bond the post to the root canal dentine. In the case of prefabricated post restorations, an amalgam or resin composite core is placed around the post to replace the coronal portion of the tooth and provide a foundation for the crown restoration.

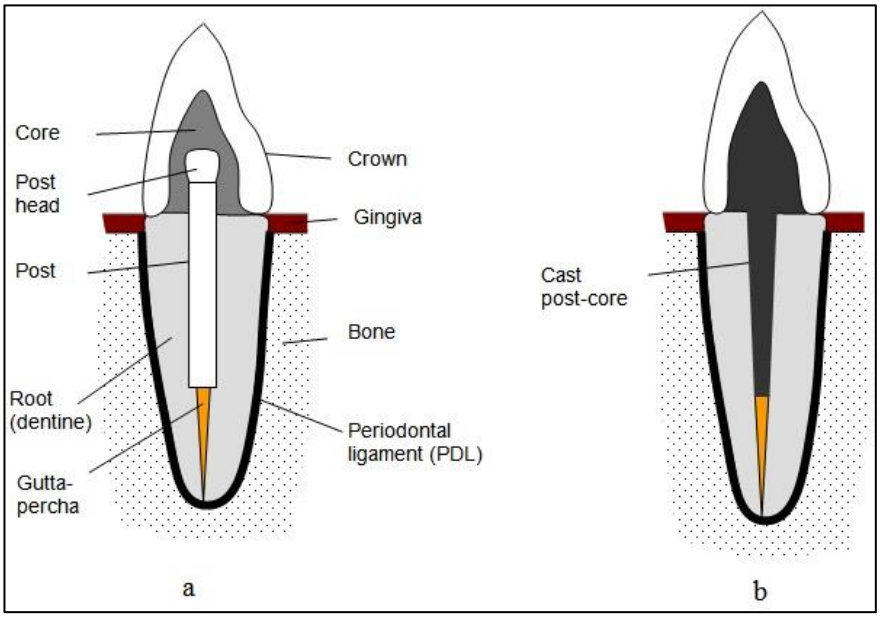

Figure 1-4 Endodontic restoration with a prefabricated post (a) and cast post-core (b) [adapted from (Pérez-González *et al*. 2011)]

1.6.1 Ideal properties of post/core materials

To date, there is still no agreement regarding which material or technique can be considered ideal for the restoration of ETT, however it is believed that the post and core system should ideally have (Fernandes *et al.* 2003; Mannocci and Giovarruscio 2016):

- **i.** adequate compressive strength to resist intraoral forces.
- **ii.** adequate flexural strength to prevent core flexion
- **iii.** modulus of elasticity similar to that of dentine
- **iv.** resistance to leakage of oral fluids at the core/tooth interface.
- **v.** ease of manipulation.
- **vi.** ability to bond to the remaining tooth structure.
- **vii.** ability to inhibit dental caries.
- **viii.** the thermal coefficient of expansion and contraction similar to tooth tissue
- **ix.** low water uptake.
- **x.** ease of retrieval when retreatment is required.
- **xi.** favourable aesthetics and radiopacity.
- **xii.** biocompatibility.

Although composite resins display the majority of the above properties, there exists need for an improved post and core system that satisfies the mechanical and clinical requirements (Liddelow and Carmichael 2016).

1.6.2 Post length, diameter and design

1.6.2.1 Post length

The post length is considered ideal when it reaches two-thirds of the length of the root, which allows for better stress distribution to the alveolar bone (Lanza *et al.* 2005). To ensure a favourable seal, the posts should be extended to the length that retains 4-5 mm of apical gutta-percha (Mattison *et al.* 1984). Numerous studies report improved retention and stress distribution when longer posts of any type are used (McLaren *et al.* 2009; Ali *et al.* 2013). However, with increasing length of the post, the risk of perforation increases and there is a decrease in the fracture resistance of the remaining root restored with a

metal post (Amarnath *et al.* 2015). Furthermore, long posts may be extremely difficult or impossible to remove when endodontic retreatment is required.

1.6.2.2 Post diameter

Post width also plays an important role in fracture resistance; the general guideline followed is that the post diameter should not exceed one-third of the root diameter at its narrowest dimension (Mou *et al.* 2009) or the post should be surrounded by a minimum of 1mm of sound dentine, especially in the apical area where the functional stresses are concentrated. However, the diameter of the post also affects flexural properties of different post systems (Seefeld *et al.* 2007). Although large diameter posts possess significantly higher maximum fracture load (Lassila *et al.* 2004), the flexural strength should be weighed against the maximum fracture load. Smaller diameter post is suggested to preserve tooth dentine minimising root fracture, but decreasing the fracture load of the post may result in failure of the restoration.

1.6.2.3 Post design

Intracanal posts can be either active or passive according to their retention mode. The use of the most retentive active post, which engages in dentine with threads, is limited to short roots in which maximum retention is needed, as it transfers more undesirable stresses to the root. While the passive post with a smooth surface, which depends on its close adaptation to the canal wall and cement for its retention, transfers stress with a lesser extent (Cheung 2005).

There are various design features and shapes of posts available: cylindrical (parallel), cylindroconical (parallel tapered), conical (tapered), double-tapered, threaded, non-threaded, smooth and serrated. It was found that parallel-sided posts are more retentive than tapered dowels (Baba *et al.* 2009). Double-tapered posts better adapt to the shape of the endodontically treated canal, thus limiting the amount of dentine tissue to be removed in post space preparation (Goracci and Ferrari 2011). Oval-shaped glass fibre posts were recently introduced for better adaptation into ovoid-shaped canals.

1.6.3 Post types

Generally, there are two main generations of post systems. Traditionally, metallic post systems include laboratory-fabricated cast post-cores and prefabricated stainless steel and titanium posts. However, in response to a need for tooth-coloured posts, several non-metallic post systems such as ceramic, zirconia and fibre-reinforced composite (FRC) posts have become available (Fig. 1-5).

Figure 1-5 Different post types: (left to right) a metallic cast post and core, a stainless steel post, a titanium alloy post, all-ceramic post (alumina), a zirconia post and glass fibre post (adapted from (Schwartz and Robbins 2004; Sahafi and Peutzfeldt 2009)

1.6.3.1 Metal cast post-and-core

The metal cast post-and-core is also known as custom cast post-and-core. The primary indication of its use is to fit the existing morphological form and diameter of the root canal, without additional preparation. Custom-fabricated posts are made of gold, silver palladium and base metal alloys, but their rigidity is a major disadvantage in adjustment and may predispose the tooth to root fracture. However, a recent clinical trial found that after 5 years of follow-up, gold alloy-based cast post-and-core and prefabricated glass fibre posts on ETT with full ceramic crown performed equally well (Cloet *et al.* 2017) but as the success of treatment depends on many factors, the outcome needs to be interpreted with caution. Other disadvantages of metal posts are their aesthetics, as the metal shows through the all-ceramic restorations, and the long placement procedure, as they require two visits and laboratory fabrication (Hochstedler *et al.* 1996). The need for placement of temporary post-crowns, while the definitive restoration is being constructed was also found to be associated with more coronal microleakage (Fox and Gutteridge 1997).

1.6.3.2 Metal prefabricated posts

Metal prefabricated posts include stainless steel, pure titanium and its alloys. Stainless steel has been used for a long time in posts fabrication. However, the high modulus of elasticity (200 GPa), sensitivity to its nickel contents and corrosion are some of the disadvantages (Goracci and Ferrari 2011). Pure titanium with a slightly lower compressive and flexural strength than Ti alloys tend to break more easily compared with stainless steel posts during removal in retreatment cases. Titanium alloys have a higher modulus of elasticity (~110 GPa) which are markedly greater than that of dentine. Ti alloys also have similar radiopacities to that of gutta-percha and sealers that make it difficult to distinguish on radiographs. However, the low corrosion and biocompatibility are the main advantages of this material (Cheung 2005).

1.6.3.3 Ceramic posts

Ceramic materials such as alumina, feldspathic and zirconia ceramics are used for the fabrication of all-ceramic posts and cores, which characterised by high flexural strength and fracture toughness, in addition to favourable aesthetics, biocompatibility and radiopacity (Purton *et al.* 2000). However, ceramic posts have a high modulus also and show poor bonding to dentine walls under fatigue (Dietschi *et al.* 1997; Hedlund *et al.* 2003).

1.6.3.4 Zirconia posts

Zirconia posts have high flexural strength, high fracture toughness, chemical stability, biocompatibility and favourable optical properties similar to that of natural teeth (Vichi *et al.* 2000). However, zirconia posts are nearly impossible to remove from the root canal when a failure occurs. Another disadvantage of zirconia posts is the rigidity, their high elastic modulus at 200 GPa causes stress to be transferred to the less rigid dentine, thereby resulting in root fractures (Guazzato *et al.* 2004).

1.6.3.5 Fibre reinforced composite (FRC) posts

FRC materials are characterised by better functional and structural design, their mechanical properties can be tailored to closely match dentine and also allows minimally invasive and adhesive techniques to preserve tooth structure (Fernandes *et al.* 2003).

The mechanical, optical, and bonding properties of these materials depend on the type of the matrix and fibre reinforcement as well as on the interfacial adhesion between them. The fibres provide strength and stiffness, while the polymeric matrix keeps the fibres together in the composite structure, protects them from the environmental harm due to elevated temperature and humidity and acts as a load transfer medium between them. Fibre reinforced composite (FRC) posts were introduced by Duret in the 1990s (Duret *et al.* 1990). In addition to root canal posts, FRCs have been effectively used in dentistry for a wide range of dental applications including removable prosthodontics, fixed partial dentures (FPD) and periodontal and orthodontic splints (Perdigão 2016).

In FRC's, the matrix may be either thermoset (cross-linked) or thermoplastic (linear). The most commonly used matrices in FRC posts are the cross-linked ones, which include multifunctional or dimethacrylate resins such as epoxy resin, bisphenol A-glycidyl methacrylate (BISGMA) and urethane di-methacrylate (UDMA). Although thermosetting polymers with highly cross-linked networks are better than thermoplastics in terms of mechanical properties, thermal stability, chemical resistant, and durability (Yan *et al.* 2014), they demonstrate poor surface adhesive properties. These polymers cannot be easily dissolved or swollen by the matrix resin of the luting cement or core build-up materials; therefore, it is difficult to obtain sufficient bond between the thermosetting matrix of FRC posts without surface treatment (Purton and Payne 1996). Another disadvantage of using fibre posts with a highly cross-linked structure is the difficulty of removal in the event a nonsurgical endodontic retreatment (Ruddle 2004).

Fibres represent the largest volume (from 40 to 65 vol.%) and contribute to stiffness and strength of the matrix and determine the load-bearing capacity of FRCs structure (Zicari

et al. 2013a). Carbon, Kevlar (p-phenylene diamine), polyethylene, and glass fibres with micron-scale diameter have been used in either unidirectional or woven orientation as reinforcements for dental FRCs. Some properties, advantages and disadvantages of these fibres are listed in Table 1-2.

Table 1-2 Properties, advantages and disadvantages of reinforcing fibres [(Mallick 2007; Khan *et al.* 2015)]

Properties					
Fibre type	**Density g/cm^3**	**Tensile modulus GPa**	**Tensile strength GPa**	**Advantages**	**Disadvantages**
Carbon	**1.78**	**230**	**3.2**	**- High strength in both tension and compression - High resistance to corrosion, creep and fatigue. - High modulus of elasticity**	**- Poor aesthetic (Black colour) - Radiolucent - Difficult manufacturing and handling techniques (Poor wettability). - Potential toxicity**
Glass (E-glass)	**2.55**	**76**	**1.5**	**- Improved adhesion to the polymer matrix - Good aesthetic (transparent appearance) - Superior mechanical properties - Biocompatibility**	**- Higher density - Low tensile modulus - Relatively low fatigue resistance - Low chemical resistance and hydrolytic instability of silica-based glass fibre.**
Aramid (Kevlar 49)	**1.44**	**124**	**2.8**	**- Relatively low density - Low weight - High tensile and impact strength - Good thermal and chemical resistance. - Biocompatibility - Superior wettability than carbon fibres**	**- High crystallinity with chemically inert and smooth surface (Need surface treatment to improve interfacial adhesion). - Aesthetic limitation (Yellow colour) - Poor polishability**
Polyethylene (UHMWPE)	**0.97**	**120**	**2.59**	**- low density - good impact resistance - Neutral colour (White) - High ductility - Superior biocompatibility**	**- Poor adhesion with the polymer matrix and thus do not give sufficient strength (need surface treatment with acids or gas plasma)**

The reinforcing ability of the fibres depends on the diameter, orientation, quantity, compatibility, and impregnation of the fibres with the matrix resin (Vallittu 1997). The wetting of the fibres by the matrix is essential for reinforcement, transmission of stresses (Perdigão 2016), water sorption and the mechanical properties of FRCs. The interfacial adhesion of the fibre/matrix depends on the interactions between the components and can either be mechanical or chemical in nature. Mechanical bonding depends on the morphology and surface texture of the fibres, while a chemical covalent bond can be

achieved by using appropriate coupling agents (Fig. 1-6). Silanation of fibres has been shown to enhance the surface wettability and improve the adhesion by forming siloxane bridges and hydrogen bonds on the fibre surface (Vallittu 1997).

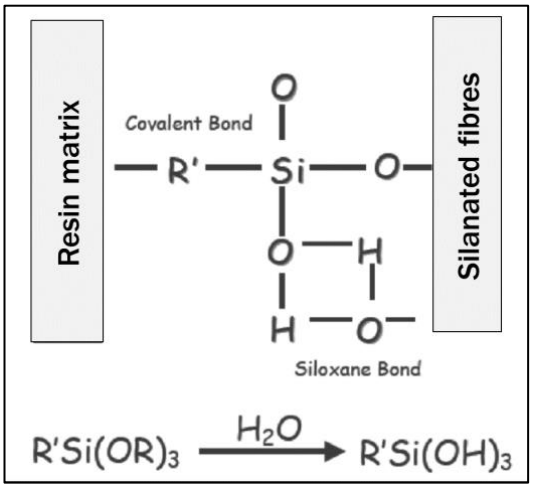

Figure 1-6 Schematic of the coupling reaction at the fibre post-matrix interface. Organosilanes with chemical formula R'-Si- $(OR)_3$ with an organic group (R') and three alkoxy groups (R): the reaction begins with hydrolysis of the alkoxide groups (R) into silanols (SiOH) that condense, forming siloxane bonds

Currently, several types of FRC posts are available and they can be categorised on the type of fibre used.

1.6.3.5.1 Prefabricated carbon fibre posts

Carbon fibre posts (Composipost, C-Post) were the first prefabricated FRC posts introduced to the market in the 1990s. The posts were made of continuous unidirectional carbon fibres embedded in an epoxy matrix (Duret *et al.* 1990). One of the most important proposed advantages with carbon fibre posts was the lower elastic modulus (more flexible) compared to metal posts, which was thought that forces would be distributed more evenly in the root, resulting in fewer unfavourable tooth fractures. However, some *in vitro* studies reported that the elastic modulus of carbon fibre posts is in fact much higher than that of dentine and similar to that of metallic and ceramic posts (Purton and Payne 1996; Asmussen *et al.* 1999) with flexural strengths usually four times higher than root dentine (Plotino *et al.* 2007a). Furthermore, the lack of radiopacity and black colouration limits their use due to poor aesthetics under all-ceramic crowns.

1.6.3.5.2 Prefabricated glass fibre posts

The high cosmetic demands prompted the development of glass FRC posts with a translucent or white appearance as an alternative to the dark carbon fibre posts. Moreover, it was claimed that the translucency would facilitate the polymerisation process of light-cured luting cements with a consequent improvement of their mechanical properties (Boschian Pest *et al.* 2002). However, the efficiency of this propagation has still to be investigated (da Silva *et al.* 2016).

Glass FRC posts are fabricated from different types of glasses that differ in their chemical composition. E-glass is the most commonly used glass, in which the amorphous phase is a mixture of a calcium-alumino-borosilicate with low alkali content (Khan *et al.* 2015). In addition, glass FRC posts can also be made of quartz fibres, which is pure silica in crystallised form.

A potential advantage of glass FRC posts is that their modulus of elasticity is close to that of dentine (Zicari *et al.* 2013a); therefore, post failure may occur before tooth fracture when force is applied. However, the mechanical properties of glass fibre post from different brands have been widely analysed in comparison with other fibre posts and the results show high variability (Table 1-3). Factors such as fibre content, fibre distribution, density, manufacturing process, structural integrity, voids and interfacial adhesion affect the mechanical behaviour of FRC posts (Khan *et al.* 2015). Furthermore, their elastic modulus and strength values depend on the post dimensions, span length as well as on the type and direction of stresses applied during testing (Kim *et al.* 2016). Glass and quartz fibre posts have been reported have similar mechanical properties as the carbon fibre posts. (Mannocci *et al.* 1999; Grandini *et al.* 2005a).

The interfacial adhesion between glass fibre and matrix is achieved via physical or chemical treatments, however water sorption is a common problem with some FRC components, especially that contain glass and silica fibres (Abdel-Magid *et al.* 2005; Khan *et al.* 2015). An aqueous and moisture environment, such as saliva in the oral

cavity, can induce “corrosion” effects on the surface of glass fibres resulting from water that diffuses through the polymer matrix (Ehrenstein *et al.* 1990). This can lead to a reduction of the mechanical properties and changes in the composite structure because the surface of the glass fibres is affected by the hydrolysis of alkali and earth alkali oxides in the glass and leaching of ions.

Table 1-3 Composition and mechanical properties of different fibre reinforced posts [adapted from (Cheleux and Sharrock 2009; Zicari *et al.* 2013a)]

Type of post	Resin matrix	Fibre	Diameter (mm)	Max. fracture load (N)	Flexure strength (MPa)	Flexural modulus (GPa)	Fatigue resistance (cycle)
Composipost (RTD,Grenobe, France)	**Epoxy resin**	**Carbon 64%**	**1.9**	**-**	**1394**	**116**	**-**
D.T. Light-Post (RTD, Grenobe, France)	**Epoxy-resin 41%**	**Quartz 59%**	**0.8-2.2**	**70-240**	**600-1000**	**10- 24**	**2,000,000**
FRC Postec Plus (Ivoclar Vivadent, Schaan, Liechtenstein)	**Dimethacrylate, 2ytterbium fluoride 47%**	**Glass 53%**	**0.8-2**	**100-175**	**600-800**	**10-19**	**1,837,138**
GC Fiber Post (GC,Corporation Tokyo, Japan)	**Methacrylate**	**Glass**	**0.59-1.6**	**75-180**	**810-1000**	**18-24**	**2,000,000**
Luxa Post (DMG Hamburg, Germany)	**Bis-GMA, UDMA, HDDMA, Ba-filler**	**Glass**	**0.63-1.5**	**70-110**	**800-850**	**17-20**	**-**
RelyX Fiber Post (3M ESPE, Seefeld, Germany)	**Epoxy-resin, zirconia filler**	**Glass**	**0.7-1.9**	**95-220**	**800-1100**	**14-22**	**-**
Para Post Taper Lux (Coltène-Whaledent Mahwaw, NJ, USA)	**Bis-GMA, UDMA, 2 HDDMA**	**Glass**	**0.65-1.5**	**50- 135**	**900-1050**	**20-26**	**-**
Evolution fibre post (Innotech, Italy)	**Epoxy resin**	**Glass**	**2.12**	**213**	**455**	**2.28**	**-**

1.6.3.5.3 Customised FRC posts

These types of fibre posts are also called “individually formed fibre posts”. Customised post-and-core systems commonly comprise the use of woven polyethylene (PE) or glass fibres to fabricate endodontic posts that are cemented directly into the root canal.

Polyethylene FRC, Ribbond (Ribbond Inc, Seattle, WA, USA), is a post made of plasma treated UHMWPE-woven fibre coated with a dentine bonding agent, which are adapted into the root canal without enlargement (manufacturer's instructions) and light polymerised in position (Belli and Eskitascioglu 2008). Ribbon fibre is biocompatible, aesthetic and translucent. It is used in combination with resin cement and an adhesive, resulting in impact strength and a suitable elastic modulus close to that of dentine (Eskitascioglu *et al.* 2002). It is believed that PE posts can create a root canal mono-block allowing for a more favourable stress distribution along the root dentine, reducing the incidence of vertical root fracture (Belli *et al.* 2011). Moreover, a previous study showed that the use of PE post to restore ETT reduces the coronal leakage when compared to stainless steel or zirconia post systems (Usumez *et al.* 2004). However, the strength of PE customised post did not approach that of a cast metal post (Baba *et al.* 2009) and the addition of small-size prefabricated post is recommended to increase the strength of PE post-core system.

A custom-made post system utilising silanated glass fibres impregnated with a semi-IPN polymer matrix (everStick®Post; Stick Tech Ltd, Turku, Finland) has been also developed with the same concept of the individually formed post. Because of its pre-curing plastic state, this post can fill the entire space of the root canal with more reinforcing fibres in the cervical portion using minimally invasive preparation and increasing the load-bearing capacity as a potential benefit of this post system (Le Bell-Rönnlöf *et al.* 2011). EverStick® FRC is based on a combination of thermoset and thermoplastic resins matrix that contains poly bis-GMA as the cross-linked phase and poly methyl methacrylate (PMMA) as a linear phase (Sperling 1994). This system, which is defined as a semi-interpenetrating polymer network (semi-IPN), has been reported to be responsible for the formation of *in situ* semi-IPN nano-interface between the matrix and the fibre improving the mechanical properties of the material (Lassila *et al.* 2004). Furthermore, the non-cross-linked thermoplastic polymer chains (PMMA) allows the diffusion of bonding and/or cement resin monomers into the linear polymer phase of post matrix

(Mannocci *et al.* 2005). However, it is thought that the compatibility of individual components in IPN-based fibre post plays a critical role in determining their thermal stability and mechanical properties (Su *et al.* 2014). Moreover, the *in situ* hand light-polymerisation of the everStick® post may also result in inferior mechanical properties (Lassila *et al.* 2005) explaining the high clinical failure rate reported (Cagidiaco *et al.* 2008a).

1.6.4 Post adhesion

Reliable retention, which depends highly on post surface characteristics, is considered to be the main concern with all kinds of posts (Jongsma *et al.* 2010). The least retention is seen with smooth surface posts, while the highest retention is observed in the threaded post, followed by the post with a serrated surface. Unfortunately threaded posts mechanically engage dentine and increase the risk of root cracks (Cheung 2005).

The development of metal primers enables adhesive placement of metallic cast and prefabricated posts with resin cement. Chemical bonding to precious and non-precious metal can be enhanced by metal primers containing proprietary monomers that simultaneously bond to the metal atoms and copolymerise with resin monomers (Tsuchimoto *et al.* 2006; Soares *et al.* 2009).

FRC posts are adhesively luted into the root canal, this fixation turned out to be more relevant for post retention compared to the post design. To increase mechanical retention, different surface features, e.g., serrations have been added to prefabricated fibre post design. However, it has been shown that these modifications could adversely affect the adhesion and flexural strength of a FRC post with an anisotropic nature (Zicari *et al.* 2013a). However, most of the currently used prefabricated fibre posts are designed to be passively retained into the root canal and have a smooth surface that restricts micromechanical interlocking with resin composites. Furthermore, their cross-linked polymer matrix is chemically inert with no interactive functional groups to bond resin cement and composite core material (Purton and Payne 1996). Therefore, different

surface treatments, of the FRC post surface, facilitating both chemical and micromechanical retention, have been employed to improve the adhesion (Mannocci *et al.* 1999; Goracci and Ferrari 2011).

Silanisation, hydrofluoric acid etching, sandblasting, hydrogen peroxide and methylene chloride (CH_2Cl_2) surface pre-treatments have applied with varying degrees of success due to different components of FRC post (Asakawa *et al.* 2013). The use of a silane coupling has been reported as the most reliable method to promote adhesion by increasing the post surface wettability and establishing chemical bonding between the methacrylate groups of the resin and hydroxyl groups of quartz and glass fibres (Aksornmuang *et al.* 2004; Goracci *et al.* 2005), However, other studies have disagreed with the efficacy of silanisation, stating that the procedure did not produce any considerable difference in bond strength (Perdigão *et al.* 2006; Ferrari *et al.* 2008b).

For better adhesion, a combination of silanisation and micromechanical treatment is recommended. Mechanical interlocking can be obtained by sandblasting with alumina particles (Choi *et al.* 2010) or chemical etching with hydrofluoric acid (Kulunk *et al.* 2012) or hydrogen peroxide (Elsaka 2013) that can remove the outer layer of resin, exposing the fibres to silanisation. However, the main problem related to these techniques is the risk of fibre damage at the surface with possible weakening effects on the stability and integrity of the posts as well as changes in post shape and fit within the canal. Another option to promote adhesion is the use of a tribochemical coating, which involves silicoating of the post surface, followed by silanisation (Radovic *et al.* 2008a).

Although all above mentioned surface treatment techniques have been established for improving post adhesion, debonding is still the most frequent failure mode of FRC retained restorations (Parisi *et al.* 2015).

1.6.5 Post radiopacity

Radiopacity is an important factor in the choice of dental posts. They should be more radiopaque than dentine and have a similar or slightly greater radiodensity than that of enamel to allow the identification of the interface between posts and tooth substrates (Rodrigues *et al.* 2014). This helps clinicians to evaluate post fitting and helps in the detection of secondary caries, marginal defects and loss of retention or post fracture (Hitij and Fidler 2013). The radiopacity of dental composite materials is determined according to the ISO protocol 4049, using a digital radiographic image in comparison with the opacity of an aluminium step-wedge. The radiopacity of the selected material is expressed in terms of the equivalent thickness of aluminium per 1 mm unit thickness. Other studies compared the grey value differences between dentine and different post materials inside the root canal of extracted teeth in dry mandibles simulating the clinical setting (Wicht *et al.* 2011). FRC posts have lower radiopacity values than traditional metal or zirconia posts hence addition of radiopaque fillers could improve radiopacity of endodontic FRC post materials (Furtos *et al.* 2016).

1.6.6 Post removal

According to recent studies on nonsurgical root canal therapy outcomes, the RCT fail in approximately 17-20% of the cases (Ng *et al.* 2011a; Burry *et al.* 2016). Moreover, diagnosis using cone beam computed tomography (CBCT) revealed a higher failure rate (26.1%) for primary root canal treatment than periapical radiographs at 1 year (Patel *et al.* 2012). In most cases a retreatment of the root canal is undertaken after removal of the post. Ideally, post systems should be easily retrievable by the clinician without weakening, perforating, or fracturing the remaining tooth structure. Many factors influence successful post removal such as material, shape and length of the post, accessibility, type of luting agent as well as the operator experience and technical resources available (Plotino *et al.* 2007b; Ebrahimi Dastgurdi *et al.* 2013). Post retrieval can be performed using a variety of techniques and instruments, including bur kits or

trephines, endodontic extractors, post pullers and the use of ultrasonic devices (Ruddle 2004).

The removal of a metallic post is difficult and may involve the removal of sound tooth structure around the post that can further weaken the root (Fernandes *et al.* 2003). Several studies have reported that ultrasonic vibration of posts facilitates their removal by breaking the cement adhesive layer (Plotino *et al.* 2007b; Braga *et al.* 2012; Aguiar *et al.* 2014). This technique offers suitable efficiency, speed and safety while preserving tooth structure.

Ceramic and zirconium posts are considered difficult and sometimes impossible to retrieve because of their rigidity (Ouml *et al.* 2010). Removal of metallic or zirconia posts by ultrasonic vibration has been found to cause rapid increases in temperature of the post and root surface and the heat generated in metal posts, even with adequate water-spray cooling, can be transferred to the periodontal ligament, causing irreparable damage (Budd *et al.* 2005).

In contrast, the removal of FRC posts is claimed to be relatively easy and rapid (Gesi *et al.* 2003; Frazer *et al.* 2008) and can be done by progressive drilling through the middle of the post with specially designed reamers (removal kits). For maximum root structure preservation and removal effectiveness, the use of specific ultrasonic tips to disrupt the composite structure with adequate magnification technique is required (Lindemann *et al.* 2005). However, retreatment of fibre posts with a highly cross-linked matrix structure and cemented with adhesive systems presents a challenge for many clinicians when RCT has failed (Soares *et al.* 2009). Additionally, a fibre post with a lower modulus of elasticity conducts vibration less efficiently and combined with improved bonding, this may reduce the effectiveness of ultrasonic in fibre post removal (Plotino *et al.* 2007b).

1.6.7 Recent development in endodontic post systems and restorative techniques

Biodegradable polymers, polyglycolic acid (PGA) and poly-lactic acid (PLLA) were extrusion-compression moulded for potential use as a screw post for primary teeth (Mizutani *et al.* 2012), which were reported to exhibit an adequate rate of the hydrolysis, but no information about their behaviour in water and its effects on mechanical properties was reported. More recently, *Hu et al*. examined the feasibility of applying a high-performance fibre, poly p-phenylene-2, 6-benzobisoxazole (PBO), as an option to develop a post material with improved flexural strength (Hu *et al.* 2014). However, a conventional epoxy resin matrix was used in post preparation, which has poor bonding ability (Purton and Payne 1996).

Other experimental FRC posts based on alkaline-resistant (AR) glass fibres and methacrylate matrix were proposed as an alternative to epoxy resin/glass fibres commercial endodontic posts. It was concluded that a high tensile strength AR glass fibre had a great potential for obtaining new glass FRC post with a good mechanical behaviour, close to other glass fibre post on the dental market (Baldea *et al.* 2015). However, the thermal and cyclic fatigue of experimental posts were not estimated to emphasise the advantage of using AR glass fibres.

To facilitate fibre post removal several different approaches have been investigated. Hi-Rem Post (Overfibers, Ferrara, Italy) was marketed with a polymer microfiber in the longitudinal axis that has been reported to require less time for removal with an endodontic nickel-titanium rotating instrument when compared with quartz fibre post removal (Scotti *et al.* 2013). In another attempt, a fibre reinforced plastic, hollow fibre post, was fabricated from unsaturated polyester matrix and a plain-woven fabric of carbon and glass fibres as a new direct core build-up method for restoration of ETT. It was claimed that by injecting the restorative material into the bottom of the root canal through the hollow post decreased the number of voids in the core and increased its bonding strength to the fibre post when compared with the conventional method (Inaba

et al. 2013). Furthermore, this design could also facilitate the removal with little tooth damage by providing a cavity in the shank of the hollow post, which serves as a guide for reaming the post out of the tooth.

Regarding the restorative techniques, the use of anatomic posts as a treatment option for the restoration of ETT with flared root canals has been reported by several studies (Marchi *et al.* 2003; Silva *et al.* 2011; Mongruel Gomes *et al.* 2014). The excessive root canal flaring combined with thin dentinal walls complicates the restorative procedure and can lead to future root fracture under normal functional stresses (Flanagan 2014). In order to avoid the extraction of flared roots, filling the root canal space with suitable restorative materials such as glass-ionomer cement (Marchi *et al.* 2003), resins composite (Barcellos *et al.* 2013), and accessory glass fibre posts (Li *et al.* 2011; Latempa *et al.* 2015) have been attempted. The anatomic post technique involves the anatomical shaping of the prefabricated fibre posts with a composite resin into the root to provide a close adaptation of the post to the root canal, reduce the resin cement thickness, and therefore, improve the retention. Some case reports highlighted the importance of this protocol as a successful treatment for weakened roots by reporting favourable clinical outcome (Grandini *et al.* 2003; Gomes *et al.* 2016).

With the improvement in adhesive technologies, a simple and efficient concept of an adhesive restoration, endocrown, has been suggested to restore endodontically treated teeth (usually posterior) with extensive coronal destruction as an alternative to post- core and crown treatment (Biacchi *et al.* 2013). Endocrown, which could be fabricated from hybrid resin composite or ceramic (Gresnigt *et al.* 2016), is a monolithic restoration bonded on ETT and using the entire extension of the pulp chamber and possibly the root canal entrances as a retentive resource instead of the intraradicular posts. In addition to the simple clinical procedure, the main advantage of endocrown is the more conservative approach with proper marginal stability that enhanced by retaining maximum enamel to improve adhesion (Fages and Bennasar 2013). This provides a good seal of the root canal opening, preventing bacterial recolonization, which in turn affects the long-term

prognosis of ETT. However, one of the major disadvantages of endocrown restorations is the high risk of fracture failure because of the absence of a metal or high-strength ceramic substructure as in conventional full-crown (Gresnigt *et al.* 2016). Although this treatment option is relatively uncommon, some short-term clinical studies present promising results in restoring function, aesthetics, and maintaining the biomechanical integrity of the compromised structure of endodontically treated molars (Bindl and Mormann 1999; Bernhart *et al.* 2009). Furthermore, finite element analyses showed that molar teeth restored with endocrowns were potentially more resistant to failure than those restored with fibre posts (Dejak and Młotkowski 2013).

1.6.8 Comparative studies of Post systems

In the literature, the post and core restorations of ETT have been extensively investigated by both *in vitro* and *in vivo* studies. A large number of laboratory studies have focused on the effect of post placement, comparing the fracture strength and failure mode of post/tooth complex using continuous or cyclic loading to represent *in vivo* conditions. The results of these studies are controversial because of the wide variety of materials and experimental design used (Schwartz and Robbins 2004), however, it can be concluded that a more favourable failure mode is clinically better than high fracture resistance. Metal posts of high stiffness promoted higher values of fracture load and higher levels of irreparable tooth fractures (Barcellos *et al.* 2013; Wandscher *et al.* 2014), whilst FRC posts with lower modulus resulted in more favourable failure modes such as post debonding or post fracture.

Data from clinical studies on the outcome of endodontic post restorative materials and techniques are presented in Table 1-4. Different failure rates for ETT restored with different post restorations have been reported and conflicting results have also been found regarding the effect of post placement and post type on the survival of ETT.

Table 1-4 Clinical studies on outcome of different post systems

Study	Post systems	Test	Results	Conclusions and limitations
Ferrari *et al.* (2007)	**A total of 985 posts placed into ETT: One carbon fibre post Two quartz fibre posts**	**Failure rate at 7-11 year period**	**A 7-11% failure rate was recorded for the three types of posts with no significant differences among them. 79 failures in total were noted; 39 due to endodontic reasons, 1 root fracture, 1 fibre post fracture, 17 crown dislodgements and 21 due to post debonding.**	**The use of fibre posts in combination with adhesive restorative materials can provide a long-term clinical success in ETT. In this study, the effects of different bonding systems and coronal restorations had not considered because of the different sample size in each group.**
Cagidiaco *et al*. (2008a)	**A total 360 premolars provided 6 groups of 60 classifieds according to the number of remaining coronal walls, each group restored with: 20 No post retention; 20 Prefabricated quartz fibre post (QFP); 20 Customised everStick® post (EV)**	**Failure rate at 3-year recall**	**The overall failure rate was 33.3%. Teeth without post showed significantly higher failure rate and largest numbers of root fractures and crown dislodgements failures. Teeth restored with customised EV post had higher failure rates (with a high frequency of post/core fractures) than prefabricated QFP (with post debonding major failure event).**	**The placement of a prefabricated or a customised post was shown to contribute significantly to the survival of ETT. This contribution was more effective for QFP than for EV. Regardless of the restorative procedure, the preservation of at least one coronal wall significantly reduced failure risk.**
Bitter *et al.* (2009a)	**A total 120 restored teeth (anterior, premolar, molar) with various numbers of residual coronal walls: 60 No post 60 Quartz Fibre Post**	**Failure rate at 3-year recall**	**The overall failure rate of no post group was 10%, whereas, in post group, the failure rate was 7%. In no-wall group, teeth without post retention revealed a significantly higher failure rate (31%) compared with teeth restored with post retention (7%).**	**Fibre post placement was efficacious to reduce failures of post endodontic restorations only with teeth that exhibited no coronal walls. The small sample size is a major limitation**
Zicari *et al.* (2011)	**A total 205 restorations in ETT: Glass fibre Posts, Composite Cores (no post), Gold alloy Posts and Cores**	**Failure rate at 3-year recall**	**The overall failure rate at 3 years amounted to 8.3%. Mode of failures: 2 root fractures, 1 endodontic failure, 3 retention loss of the post/core and 1 post fracture.**	**Both cast gold and composite post-and-core systems performed well clinically. Longer follow-up times are needed to detect possible significant differences.**
Sterzenbach *et al.* (2012a)	**A total 91 restored teeth: 45 titanium post (TP) 46 glass fibre post(GFP)**	**Failure rate at 7-year recall**	**Overall survival rate of both post restorations was 92%. No difference in failure rates between GFP (9.8%) and TP (6.5%). The failure modes for GFP: 3 root fracture and 1 core fracture, while for TP: 3 endodontic failures.**	**Irrespective of the post material, the used of adhesively luted prefabricated posts in severely destroyed ETT achieved high long-term survival rates. The small sample size with a relatively high drop-out rate (24%) is a major limitation.**

Naumann *et al.* (2012)	**A total of 157 post restorations (anterior and posterior teeth):** **Three different glass FRC post systems were used consecutively**	**Failure rate at 10-year recall**	**A high failure rate of 46%.** **In total, 55 failures were observed with the most frequent ones being post fracture, loss of post retention (both n = 17), endodontic problems (n = 7), and those resulting in tooth extraction (n = 10)**	**Tooth type, type of final restoration and the lack of remaining coronal walls were found to be significant predictors of failure rates in ETT with glass fibre posts.** **The major limitation of this study is the small sample size with a high drop-out rate (27%).**
Sarkis-Onofre *et al.* (2014a)	**A total 72 restored teeth with no remaining coronal wall:** **37 Glass fibre post (GFP)** **35 Cast-metal post (CP)**	**Failure rate at 3-year recall**	**No difference in failure rates between GFP (8.1%) and CP (2.9%).** **The failure modes for GFP: 2 debonded and 1 root fracture, while for CP: 1 root fracture.**	**Glass fibre and cast metal posts showed similar clinical performance in teeth with no remaining coronal wall after 3 years of follow-up. However, a large sample size and longer follow-up periods are needed to detect possible differences between post systems over time.**
Parisi *et al.* (2015)	**114 Quartz fibre posts restored teeth (anterior and posterior)**	**Failure rate within mean period of 5.8 ± 1.3 year recall**	**14.1 % failure rate.** **Post debonding was the most frequent failure mode, followed by endodontic failure. No root fractures were recorded.**	**The high prevalence of debonding failures observed suggests that dentine adhesives and cementation techniques need to be improved. The study analyses the influence of tooth location (anterior or posterior) rather than tooth type as a variable.**
Cloet *et al*. (2017)	**A total 203 restorations divided into:** **1- prefabricated glass fibre posts** **2- custom-made glass fibre posts** **3- composite cores without posts** **4-cast gold alloy posts-and-core** **All followed by full ceramic restorations**	**Failure rate at 5-year recall**	**Overall failure rate was 14.8%.** **No significant differences between groups.**	**Cast gold and composite post-and-core systems on teeth with ceramic full restorations provided performed equally well.**

1.7 Bonding of post restoration

1.7.1 Dental adhesives

Adhesion of restorative materials to tooth tissue and to fixtures such as crowns in dentistry is based on creating a microporous surface that allows the ingress of the bonding agent or adhesive to enable compatible surfaces that can be made to adhere.

All dental adhesive systems comprise of an etchant, primer and bond that can be classified into different categories according to the number and combination of the clinical steps (Van Landuyt *et al.* 2007). The most commonly used etchant is 30-40% phosphoric acid. The primer is composed of hydrophilic monomers, oligomers, or polymers in a volatile solvent, such as acetone, ethanol, or water. It enhances the wettability of the surface and facilitates the penetration of the resinous monomers into the intertubular dentine and dentine tubules after solvent evaporation. The adhesive contains the hydrophobic part of the system that allows coupling with the resin based composites or cement. At this step, an acid resistant hybrid layer is created that contains resin, collagen and hydroxyapatite (Nakabayashi *et al.* 1982). Adhesive monomers including cross-linkers and functional types are considered as the key components of the dentine bonding agents. Resin monomers such as bis-GMA, UDMA and TEGDEMA, are hydrophobic in nature similar to monomers found in resin-based composites materials and have two or more polymerisable groups (–C=C– or vinyl-groups) to form cross-linked polymers and enhance the mechanical strength for bonding agents. Functional monomers usually have a hydrophilic functional group that exhibits affinity to the dentine surface and one polymerisable group to co-polymerise with the cross-linkers monomers and/or the resin-based composites (Van Landuyt *et al.* 2007). The acidic functional monomers such as 4-META, 10-MDP and phenyl-P have the ability to demineralise dentine by releasing protons in aqueous solutions. Furthermore, some monomers are also capable of releasing fluoride (eg. PEM-F) (Han *et al.* 2006) or impart an antimicrobial property (MDPB) (Imazato *et al.* 1994).

1.7.2 Bonding to root canal dentine

The bonding protocols to root canal dentine are usually similar to those employed to bond the coronal restorations. However, bond strengths to root canal dentine are generally lower compared to those to coronal dentine (De Goes *et al.* 2007; Marques *et al.* 2008), which remains a clinical challenge due limited access, visibility, moisture control, irregular structures of secondary dentine and reduced number of dentinal tubules in the apical third of the root (Mjor *et al.* 2001). The presence of thick smear layer with remnants of plasticised gutta-percha and sealer created either by endodontic instruments or by post space preparation (Gomes *et al.* 2016) and possible incompatibilities between resin cements and dentine adhesives (Tay *et al.* 2004) also contribute to the challenge. While the negative effects of above factors may be minimised, the extremely high configuration-factor C (the ratio of the bonded to the unbonded surface area) of the endodontic cavity cannot be avoided (Davidson *et al.* 1984). This leads to sufficient stresses during polymerisation to cause debonding of the luting material from root canal dentine, thereby decreasing retention and increasing microleakage (Tay *et al.* 2005).

Dentine bonding is commonly evaluated by measuring *in vitro* bond strength (De Munck *et al.* 2012), which is generally measured either by micro-tensile strength or by pull-out and push-out methods. However, the bond strength value cannot be considered as a material property. The data recorded depend mostly upon experimental factors such as composite type, stress rate, sample size and geometry, and test method. The push-out test simulates the clinical condition better than the micro-tensile techniques in measuring the bond strength of adhesively luted fibre posts (Goracci *et al.* 2004). However, it was suggested that applying push-out adhesion test on thick root sections may develop highly non-uniform stress at the bonding interface resulting in relatively low bond strength level (Ngoh *et al.* 2001).

In conjunction with bond strength evaluation, the location of the failure within each sample (failure mode) should also be assessed. The failure mode can be classified into

three main categories: cohesive within the test material or tooth substrate, adhesive within the adhesive interface and mixed failure, which presents as a mixture of both adhesive and cohesive failure within the same failed surface (Scherrer *et al.* 2010). Stereomicroscopes at high magnification are usually used to determine failure, however, scanning electron microscopy (SEM) or tandem scanning microscopy (TSM) provide more accurate decisions on adhesive and mixed failure modes (Scherrer *et al.* 2010). A strong relationship between the failure pattern and the bond strength value exists; the higher value of bond strength, the higher event of cohesive failure (Leloup *et al.* 2001). The morphological analyses of dentine/adhesive interface created by different adhesive strategies inside the root canal have been studied using confocal laser scanning microscopy (CLSM) and SEM to help predict the durability of these adhesive systems (Bitter *et al.* 2009b; Profeta *et al.* 2012; Bitter *et al.* 2014). Other microleakage and nanoleakage test methods, using different organic dyes as tracers, are also applied to evaluate the sealing ability of dental adhesive (Sano *et al.* 1995). The main problem of microleakage evaluation is the qualitative nature of this method and the results obtained in each study group differ only slightly, rendering interpretation of the results difficult and reducing the sensitivity of the test (Castelnuovo *et al.* 1996).

Literature findings highlight the conflicting *in vitro* results obtained when bonding to root canal dentine (Bindl and Mormann 1999; Zicari *et al.* 2008; Radovic *et al.* 2008b; Mazzoni *et al.* 2009). The “self-etch” system is considered to be less technique sensitive for root canal adhesion procedures (Van Meerbeek *et al.* 2011) whereas, analysis of the adhesive interface demonstrate thicker and more uniform hybrid layers with considerably more resin tags for “etch-and-rinse” approach compared to “self-etch” (Bindl and Mormann 1999; Bitter *et al.* 2004). However, the durability of bonding to root canal is closely related to the intrinsic susceptibility of the hybrid layer components to degradation (Breschi *et al.* 2008). Although the exact mechanism of the degradation is not fully understood, It is believed that the more hydrophilic the dental bonding, such as in self-etch adhesive, the higher is the water sorption and plasticisation of polymers (Reis *et al.*

2013), which leads to the formation of a permeable hybrid layer interface with weaker mechanical properties (Dündar *et al.* 2011a) and subsequent drop in bond strength over time (Marchesi *et al.* 2013). Dentine-derived MMP's (matrix metalloproteinases) are also thought to contribute to the degradation of collagen matrices in aged resin-dentine bonds (Breschi *et al.* 2009). In addition, as dual-cured resin cements are usually used for post cementation, a chemical incompatibility between acidic monomers from simplified self-adhesive systems and self-curing components of these cements has been reported (Arrais *et al.* 2009) which may contribute to the variation. The amine co-initiators in dual-cured materials undergoe an acid-base reaction with the acid-modified monomers.

1.8 Luting of post restorations

An adequate retention of intracanal posts plays a significant role in the outcome of the restoration (Goracci and Ferrari 2011). Successful bonding decreases post wedging within the root canal and reduce the need for dentine removal allowing the use of shorter and thinner posts which can decrease the susceptibility to tooth fracture (Pontius *et al.* 2002). Furthermore, the ability of luting agents to seal the tooth/restoration interface is important to prevent bacteria leakage that may lead to secondary caries.

The requirements of the luting materials used for posts cementation include effective bonding through mechanical interlocking and adhesion, high strength and fracture toughness, ease of use and manipulation (long working time and short setting time), lower solubility, low film thickness (viscosity), appropriate radiopacity and biocompatibility and the ability to inhibit dental caries (Sakaguchi and Powers 2012). The most common luting agents used for intracanal posts cementation are zinc phosphate, glass ionomer and resin-based cements. Resin cements are preferred for fibre post cementation, as an increase in post retention (higher bond strengths) and higher fracture resistance is expected when compared to conventional zinc phosphate (Sen *et al.* 2004) and glass-ionomer cements (Macedo *et al.* 2010). Furthermore, it has been reported that the adhesive luting of the posts with a dentine adhesive system and a resin cement

reduces the coronal leakage (Mannocci *et al.* 2001a), which is considered the major problem in successful endodontic treatment.

1.8.1 Resin based luting cements

Resin-based luting cements are low-viscosity composite materials containing dimethacrylate and oligomer monomers matrix with filler content adjusted to allow for low film thickness. High and low-molecular-weight monomers such as Bis-GMA, UDMA, Bis-EMA and TEGDMA are combined to achieve a high degree of polymerisation with low volumetric shrinkage. Silanated radiopaque glass (barium, strontium, or zirconia) and silica fillers are usually used in volume fractions between 30 to 60% with average particle sizes between 0.5- 8.0 µm (Sakaguchi and Powers 2012).

Due to limited access of light in the full post space, light cure materials are not recommended, since even with the use of translucent posts, the amount of light reaching the deeper regions may not be enough to efficiently polymerise the luting material (Salomao *et al.* 2015). The use of self-cure cements is also advantageous for the purpose of polymerisation stress relief due to the slower setting reaction in comparison to light-cured materials (Feng and Suh 2006). However, the limited working time of self-cured cements may complicate clinical handling. Therefore, dual-cure materials combine the advantages of both auto and light-polymerisation cements and provide the most reliable option for achieving secure polymerisation in deep parts of the canal and better clinical handling as it allows extended working time. Dual-curing resins generally exhibit higher polymerisation efficacy and mechanical properties (Hofmann *et al.* 2001; Arrais *et al.* 2008; Kournetas *et al.* 2011). However, there is an ongoing debate on the effectiveness of photo-polymerisation of dual-cured cements; it has been stated that self-curing mechanism alone is insufficient to achieve maximum hardening (Hashimoto *et al.* 2003) because of the limited effect of the chemical activator on the polymerisation of some dual-cured cements (Watson 1997).

Resin luting systems can also be divided according to the bonding strategies into conventional resin cements, used after an adhesive (etch-and-rinse or self-etching primers) application and the self-adhesive resin cements, designed to adhere to the tooth substrate without adhesive application. The main compositional difference of these self-adhesive resin cements is the presence of acid-functionalised methacrylate monomers, which include predominantly either carboxylic or phosphoric acid groups to achieve demineralisation and bonding to the tooth substrate in a simplified single-step adhesive approach (Ferracane *et al.* 2011).

Despite encouraging results on the use of self-adhesive resin cements for bonding fibre posts in terms of improving bond strength and reducing nanoleakage compared to multistep resin cements (Sarkis-Onofre *et al.* 2014), researchers have suggested that pre-conditioning the root canal dentine surface with phosphoric acid might be advantageous with respect to dissolving the thick smear layer (Bitter *et al.* 2004) to improve the bond strength of self-adhesive resin cements (Pisani-Proenca *et al.* 2011). A recent study reported that the use of these cements as core build-up underneath glass ceramic crowns is not recommended because of their hygroscopic expansion that might damage the crowns (Sterzenbach *et al.* 2015). The current evidence indicates that the most reliable results in fibre post luting are obtained by etch-and-rinse adhesives in combination with dual-cure resin cements (Dietschi *et al.* 2008; Ferrari *et al.* 2008a).

1.8.2 One-stage procedure: post-and-core system

A current trend is the use of dual-cured resin composite materials with high filler content to adhesively restore the structurally compromised ETT (Naumann *et al.* 2011). These materials have different viscosities and superior mechanical properties than those of resin cements to be used for core build-up as well as for fibre post luting in a one-stage procedure “post-and-core system” (Boschian Pest *et al.* 2002). This reduces technique sensitivity, the time required to complete the procedure and adverse effects that result from possible incompatibility between cement and core material (Bindl and Mormann 1999).

In noncircular, wide or flared root canal, close fitting of a prefabricated post is not achievable. Therefore, the use of current resin cements with a lower modulus of elasticity may create a zone of high stresses especially when a thick layer of cement is present, which becomes the weakest part of the system (Boschian Pest *et al.* 2002). The use of core materials in fibre posts cementation has been described as a secondary endodontic monoblock (Fig. 1-7). This results in mechanically homogeneous restorations with uniform stress distribution, which better preserves the weakened tooth structure (Tay and Pashley 2007). However, a previous study reported possible negative effects on the luting ability of core build-up materials for cementation fibre posts due to the higher filler content in relation to polymerisation stress development, bond strength, and microleakage (Ferrari *et al*. 2009). As mentioned earlier, the high configuration factor (C-factor) of the endodontic cavity (Feilzer *et al.* 1987) and the unfavourable application conditions may affect the bonding ability within the root canal. The high contraction stress, gaps and voids within the cement interface could lead to bonding failure and consequently, increase different adhesive strategies to root canal dentine microleakage (Grandini *et al.* 2004; Tay *et al.* 2005).

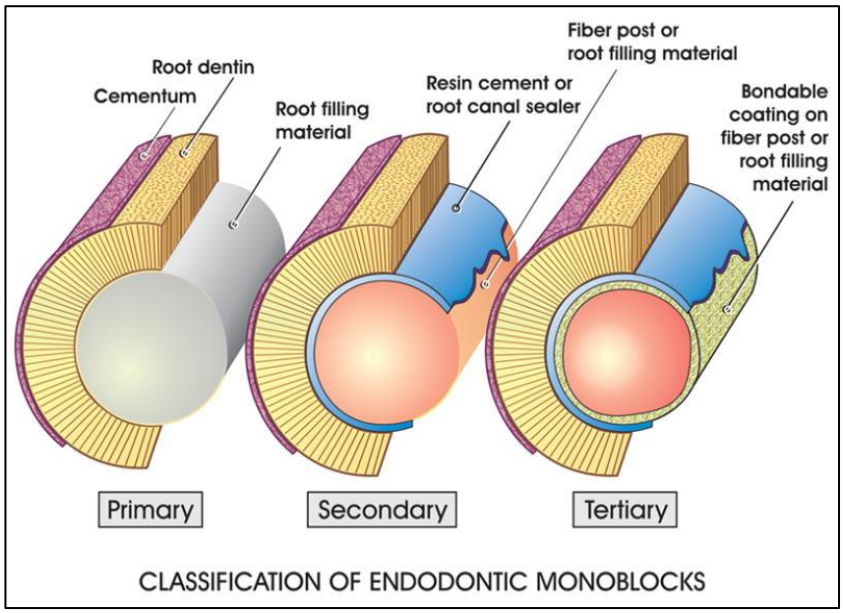

Figure 1-7 Classification of root canal monoblocks. The number of interfaces between the fibre post/root filling material and the root dentine is used to classify the endodontic monoblock into primary, secondary and tertiary monoblock [adapted from (Tay and Pashley 2007)]

Other parameters such as the viscoelastic and rheological properties of luting materials have also a direct effect on the contraction stress and microleakage. These properties are influenced by the formulation of the monomer and filler (Lee *et al.* 2006). While a positive correlation between filler content and shrinkage stress has been demonstrated (Condon and Ferracane 2000), assuming a relevant effect of material rigidity on stress development, it has also been reported that highly filled resin composites with high modulus of elasticity exhibit lower contraction stress and lower volumetric shrinkage (Baroudi *et al.* 2007). The composition of the resin matrix influences both the viscoelastic and rheological properties (Ellakwa *et al.* 2007). For example, the incorporation of low molecular weight monomers can enhance the flexural properties and lower viscosity that help in stress relief via resin composite flow relaxation (Davidson and De Gee 1984; Labella *et al.* 1999).

The composition of the many post-and-core-systems available on the market differ, hence these differences could affect their bonding ability when testing with different adhesive approaches. It has been concluded that, in comparison to conventional resin cements, luting with core material significantly increase the bond strength of fibre post restorations when bonded with an etch-and-rinse (Mazzoni *et al.* 2009) or a self-etch (Rödig *et al.* 2010) adhesive system. However, a comparable bond strength for a post-and-core material, when combined with etch-and-rinse adhesive has also been reported (Sterzenbach *et al.* 2012). Regarding the clinical performance of these systems, a prospective study reported that after an observation period of 4 years, the luting material did not influence the failure risk of ETT and similar results are achieved with self-adhesive resin cement and resin core material (Juloski *et al.* 2014).

1.9 Core materials

A core build-up is a restoration constructed to substitute the missing coronal tooth structure, providing the foundation for the final restoration (Cheung 2005). The core should possess features described in section 1.6.1 to provide satisfactory strength and resistance to withstand the masticatory stress during the function for prolonged periods. In the cast post and cores, the core is made with the post directly, whilst, prefabricated posts are used in combination with a restorative build-up material which is formed after cementation of the post. Glass ionomer cement (GIC), composite resin, and amalgam are the most commonly used core materials.

Although the new generation of resin modified GICs have resulted in favourable characteristics in terms of inherent adhesion, fluoride release and good clinical success, some studies (Cho *et al.* 1999; Bonilla *et al.* 2000) report inadequate strength, fatigue and wear resistance for core build-up applications (Lang *et al.* 2003; Sidhu 2011).

Amalgam has traditionally been used as a build-up material for a full coverage crown or as the definitive restoration. Due to its physical and mechanical properties (Lang *et al.* 2003) that are associated with long-term success, some clinicians still have confidence in amalgam application. However, amalgams unpleasant colour, prolonged setting, poor adhesion and the potential concern about mercury limit its use as a core material in recent years (Naumann *et al.* 2016).

The advances in composite technology and new bonding agents for resin based composites make them the most popular core build-up material. Light or dual-cure resin composite is commonly used to build-up a core filling before subsequent crown coverage. The ability to bond to tooth structure, proper seal, satisfactory mechanical properties, good aesthetics and easy handling are some of the advantages of resin composites (Mannocci and Cowie 2014). On the other hand, some of the negative features of resin-based composites such as polymerisation shrinkage (Rueggeberg *et al.* 2000), voids in the build-up filling, hydroscopic expansion and incompatibility affect

their clinical performance (O'Keefe and Powers 2001). Another major concern, which remains, is the susceptibility to bacteria colonisation. Biofilm growth is associated with secondary caries that develops after interfacial debonding of the restorative composite from the tooth structure. Composite resin restorations accumulate biofilm more than other restorative materials, such as amalgam (Svanberg *et al.* 1990; Beyth *et al.* 2007). This is mainly because of the lack of antibacterial activity of dental resin composite (Goldberg 2008).

1.10 The final restoration

As a part of treatment, many of ETT with or without post require cast restorations (crown coverage) such as gold, metal-ceramic and all-porcelain crowns. Although these restorations need extensive tooth preparation, they can provide the ETT with the desired protection (Aquilino and Caplan 2002). In literature, the relation between crown placement and survival rate of ETT has been assessed with a mixed opinion (Mannocci *et al.* 2002; Salehrabi and Rotstein 2004; Skupien *et al.* 2016). However, there is evidence that show cast restorations on posterior teeth increase tooth survival after root canal treatment (Ng *et al.* 2011b).

1.11 Failure of endodontically treated teeth with post restoration

The quality of root canal treatment and quality of the coronal restoration contribute equally in the clinical success of ETT (Gillen *et al.* 2011). The chances of healing of apical periodontitis increase with both adequate root canal filling and restorative treatment with respect to the integrity, seal and retention of the coronal restoration.

1.11.1 Failure of post/core restorations

The quality of the coronal restorations has been assessed both clinically and radiographically and several criteria are used to define failures such as the loss of post or crown retention, cracks, fractures (root or post), marginal fitness and recurrent caries. Failures may be associated with stresses from occlusion and also with post material or fabrication defects (Vallittu 2016).

From the material perspective, most laboratory and clinical research have shown that most of the failures occurring in high modulus posts such as metals are catastrophic root fractures (Ferrari *et al.* 2000; Wandscher *et al.* 2014). One of the reasons for root fracture is the uneven distribution of stresses that are transmitted directly to dentine causing potential root cracking and fracture. Another reason may be the weakness of root canal walls after endodontic treatment and post space preparation (Soares *et al.* 2012).

For FRC post restorations, longitudinal clinical studies report a relatively higher failure rate (Table 1-4). The findings of these studies propose that the lower elastic modulus decreases the cause of failure by root fracture but increases the debonding of the restoration interfaces, which is the most frequent failure mode in fibre posts (Cagidiaco *et al.* 2008b; Rasimick *et al.* 2010).

Theoretically, in order to provide a better stress distribution and fewer fractures, the post should flex together with the tooth during the function. However, having a “flexible” post allows movement of the core, resulting in subsequent bacterial microleakage and recurrent caries (Musikant *et al.* 2001; Cagidiaco *et al.* 2008b). This is especially important when a small diameter post is used. In severely broken down root canal treated teeth, as the post is considerably thinner than the tooth, a greater stiffness is needed to enhance the ability to carry the load and consequently reduce the tensile stress at the crown margin (Schwartz and Robbins 2004). Such failures could be attributed to the low elastic modulus of some fibre post types, particularly those made of E-glass fibres (Lassila *et al.* 2004; Baldissara *et al.* 2006; Durmuş and Oyar 2014).

The susceptibility of glass fibre posts to chemical, thermal and mechanical fatigue is believed to lead to an irreversible reduction in mechanical properties, which increases the risk of debonding (Mannocci *et al.* 2001b; Lassila *et al.* 2004). The polymer matrix, glass fibres, and interface between them can undergo degradation in a wet environment in service (Stewardson *et al.* 2010a; Barbizam and White 2014). Epoxy resins have long been known for their moisture absorption, followed by plasticisation and degradation

(Lee and Peppas 1993). The surface of the glass fibres is also affected by the hydrolysis of alkali in the glass and leaching of ions (Vallittu 2014) with a potential deteriorative effect of water on matrix/fibre interfacial adhesion through re-hydrolysis of silane coupling agent (Papacchini *et al.* 2006). Without adequate adhesion, fibres act as voids in the resin matrix, thereby weakening the FRCs. The difference in thermal expansion coefficients between these two components could also have an effect explaining the significant decrease in flexural strength following thermo-cycling (Mannocci *et al.* 2001b; Barbizam and White 2014). Glass fibre posts have been shown to undergo a 10% and 18% reduction in flexural modulus and strength respectively, on thermo-cycling (Lassila *et al.* 2004).

The higher number of clinical failures could be attributed to the longer period of clinical service of the fibre posts. Several *in vitro* studies showed that mechanical fatigue, that occurs clinically, contributes to the reduction in mechanical properties of FRC posts after ageing (Drummond and Bapna 2003; Grandini *et al.* 2005a; Cheleux and Sharrock 2009). A significantly lower (30%–38%) flexural strength of FRC posts for cyclic versus static loading was reported (Drummond and Bapna 2003), consequently, the posts become more flexible and stress on the post-resin-dentine interfaces, result in post-core debonding.

This, in combination with poor adhesion of highly cross-linked polymer matrix fibre posts in resin composite luting cements or core-built-up materials, provides a mechanically unstable tooth-restoration system. Furthermore, overloading of the restored tooth with a crown or other extra-coronal attachment may result in post fracture, another common frequent failure mode in fibre post restorations (Naumann *et al.* 2012). The fracture resistance of a fibre post depends on the flexural fatigue limits of the material from which it is fabricated, thus appropriate fibre packing with enhanced matrix/fibre bonding is important (Drummond and Bapna 2003).

1.11.2 Failure of root canal treatment

Failure of RCT can be attributed to technique related problems during treatment, but the major causes of failure of both inadequate root filling and well-filled root canals are persistent or secondary infections (Siqueira 2001). Studies have demonstrated that microorganisms may survive in certain parts of the root canal space that remain untouched during chemo-mechanical preparation, regardless of the technique and instruments used, such as dentinal tubules or lateral canals (Sjögren *et al.* 1997).

Obturated root canals may also be contaminated from the oral cavity, for example following post space preparation undertaken without isolation with a rubber dam, delay in placing the coronal restoration, leakage through the temporary or permanent restorative materials and fracture of the coronal restoration and/or tooth. In such circumstances, bacteria may invade and recolonise the root canal system with the possibility of reaching the periradicular tissues and thereby jeopardise the outcome of RCT (Siqueira *et al.* 1999). The ability of bacteria to withstand nutrient depletion via several regulatory systems is important for their survival in such conditions.

1.11.2.1 Endodontic biofilm

Dental biofilm can be defined as a sessile microbial community of aggregated microorganisms that bind to a surface or to each other embedded in an extracellular polymeric matrix (Donlan and Costerton 2002). The microflora of the oral cavity is diverse, and over 700 different bacterial species are detected in the healthy human mouth (Zijnge *et al.* 2010). These bacteria may pass through the root canal space by caries, trauma, defective filling, or via the periodontal pocket formation. The infected or necrotic pulpal tissue provide selective habitats for the creation of complex microbial biofilms with a great predominance of obligate and facultative anaerobic bacteria (Siqueira 2002). The endodontic biofilm within the root canal and the organised bacteria show higher resistance to antimicrobial agents and host defence mechanisms when compared with a planktonic population (Siqueira 2001). The combined effects of both

bacterial infection and the immune response they induce may result in a subsequent development of periapical disease (Wang *et al.* 1997).

Studies have been carried out using culture and/or molecular methods for the identification of microbial taxa of endodontic infections. Their analysis revealed that all the detected bacterial taxa belong to one of the following phyla: *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, Spirochaetes, Synergistes*, TM7 and SR1 (Siqueira and Rôças 2009). The microflora associated with failed treatment is different from that of primary root canal infections (untreated teeth) (Sundqvist *et al.* 1998). In the primary endodontic lesion, the microflora is typically mixed, in which Gram-negative anaerobic rods are dominant, whilst the microflora in failed cases is usually limited to a small number of predominantly Gram-positive microbial species; facultative anaerobes, especially *Enterococcus faecalis*, is the most commonly isolated microorganisms (Molander *et al.* 1998).

1.11.2.2 Selection of bacteria from growing biofilm

Previous studies have clearly shown that the Gram-positive taxa including representatives of *Propionibacterium, Staphylococcus, Actinomyces, Lactobacilli, Streptococci, Gemella, Micrococcus, Rothia* along with *E. faecalis* are the most predominant bacteria isolated from teeth with failed endodontic treatment (Sjögren *et al.* 1997; Niazi *et al.* 2010).

E. faecalis is an opportunistic pathogen most frequently isolated from persistent endodontic infections and has been demonstrated to be extremely resistant to several medicaments, including calcium hydroxide (Evans *et al.* 2002) and to a wide range of antibiotics (Kayaoglu and Ørstavik 2004). Owing to its considerable virulence factors including gelatinase production and the presence of adherence dominant gene-enterococcal surface protein, *E. faecalis* has the ability to form biofilms making its eradication by conventional means extremely difficult (Mohamed and Murray 2005). Another virulence factor of *E. faecalis* is the production of hyaluronidase, which helps in

obtaining energy from dentine hyaluronan and facilitates their invasion into the dentinal tubules. The role of *E. faecalis* as the initial colonisers in biofilm formation has been suggested previously because of its ability to colonise and persist in the root canal without the support of other microorganisms (Sobrinho *et al.* 1998). Therefore, the intrinsic resistance of *E. faecalis* to withstand the harsh environment of the root canal makes it a suitable microbe to investigate the efficacy of different endodontic medicaments, disinfectants and antimicrobial agents (Dahlén *et al.* 2000; Eddy *et al.* 2005).

Different species of streptococci are also dominant colonisers of failed root canal cases. The high occurrence of streptococci has been widely reported in previous studies (Molander *et al.* 1998; Siqueira and Rôças 2004) with *Streptococcus mutans*, a facultative anaerobic bacterium also identified (Molander *et al.* 1998; Pinheiro *et al.* 2003). *S. mutans* virulence factors include its ability to synthesise adhesive glucans (Xiao and Koo 2010) and generate acids that result in the demineralisation of dental tissues, thereby initiating dental caries (Tanzer *et al.* 2001). Furthermore, it has been demonstrated that *S. mutans* gains entrance into the dentinal tubules of open root canals (Kouchi *et al.* 1980) and is able to bind to collagen that may facilitate bacterial adhesion to exposed dentine and subsequently tissue penetration (Love and Jenkinson 2002).

Propionibacterium acnes has been identified among the most predominant organism in the microflora of endodontic infections (Chavez de Paz *et al.* 2004; Niazi *et al.* 2010). *P. acnes*, a non-spore-forming, Gram-positive anaerobic or aerotolerant rod, is found in the microflora of the large intestine, conjunctiva, and external ear canal and make up approximately half of the total skin microflora (Tancrede 1992). Traditionally, *P. acnes* is considered to be relatively non-pathogenic, but an increasing number of reports have noted this species as an opportunistic pathogen responsible for a wide range of infections and inflammatory conditions. It has been shown that several *P. acnes* infections such as osteomyelitis (Abolnik *et al.* 1995), endophthalmitis (Benz *et al.* 2004), endocarditis (Günthard *et al.* 1994) and others are usually accompanied with trauma and

surgery as predisposing factors. In addition, it has a well-established role in infections related to medical foreign-body implants, such as intraocular lenses (Winward *et al.* 1993), orthopaedic implants (Sampedro *et al.* 2009), silicone implants (Ahn *et al.* 1996) and prosthetic heart valves (Lazar and Schulman 1992). Recent studies by Niazi *et. al* suggested that *P. acnes* isolates from the primary and refractory endodontic lesions might be the result of nosocomial infections occurring at the time of the RCT (Niazi *et al.* 2010; Niazi *et al.* 2016).

1.12 Antibacterial activity of restorative filling materials

The antibacterial activity of resin composites and dentine bonding agent is an important property to prevent the harmful effects caused by bacteria. The antibacterial effects of resin composites are mainly relevant to inhibition of biofilm formation on the surface of the materials or to direct contact killing of microbes, while, for dentine bonding agents, their antibacterial effects are discussed in terms of disinfection of the cavity as well as inactivation of bacteria which invade the adhesive interface by microleakage. In addition to microleakage, complete removal of bacteria from cavities during treatment is difficult, in particular in the context of a minimally invasive approach (Imazato *et al.* 2006).

In general, commercially available composites and simple adhesive systems have been demonstrated to possess no or very little antibacterial activity (Imazato *et al.* 2003; Farrugia and Camilleri 2015). Moreover, it has been reported that the resin components of the composite may contribute to the growth of some cariogenic species (Mjor and Toffenetti 2000). Some adhesive systems containing glutaraldehyde are claimed to have effective antibacterial activity (Meiers and Miller 1996); however, this property may be reduced significantly or lost when contacting dentine substrate or after curing (Schmalz *et al.* 2004). Therefore, imparting sustained antimicrobial properties into these materials would influence the extent of micro-leakage, prevent secondary caries (Pereira-Cenci *et al.* 2013) and help in suppressing residual infection, increasing the survival of the restored teeth (Imazato *et al.* 2006).

1.12.1 Introduction of antibacterial agents in restorative materials

Several attempts to provide antibacterial restorative materials have been made, which involve the addition of different antibacterial agents into the resin and/or filler components. Based on the release profile, the antibacterial materials can be classified into agent-releasing materials or non-agent-releasing materials.

1.12.1.1 Incorporation of soluble and/or low molecular weight antibacterial agents

Various soluble antimicrobial agents have been added into resin matrices, which are released in a wet environment over the time inhibiting bacterial growth. Examples are antibiotics such as ciprofloxacin, minocycline and metronidazole, fluoride, chlorhexidine, zinc and silver ions (Leung *et al.* 2005; Burke *et al.* 2006; Fan *et al.* 2011; Hojati *et al.* 2013; Prabhakar *et al.* 2013). However, the release rate of these agents may greatly reduce after a short period, and long-lasting effectiveness cannot be anticipated. Moreover, it is clear that agents release produces a porous structure in the restorative materials, and their mechanical properties and bonding ability are adversely affected over time (Jedrychowski *et al.* 1983; Imazato *et al.* 2003).

The addition of antibiotics mixture increases the antibacterial activity of GICs. However, the higher concentrations exhibited lower material compressive strength and reduce bond to dentine (Prabhakar *et al.* 2013). Antibiotics are usually not recommended because of the resistance development problems.

Fluoride and metallic ions release have been shown to contribute to the antimicrobial activity of resin modified glass ionomer cements (RMGIC) (Loyola-Rodriguez *et al.* 1994; Duque *et al.* 2005). Other authors claim that the low initial pH during setting may have better effects than fluoride release (Herrera *et al.* 2000; Hotwani *et al.* 2013). The incorporation of fluoride in composite formulations has been studied previously and conflicting results on its clinical benefit have been reported (Wiegand *et al.* 2007;

Ferracane 2011). Higher concentrations, however, are needed than with antibiotics or chlorhexidine to be effective (Xu *et al.* 2010).

Chlorhexidine, which is a broad-spectrum and widely used antimicrobial agent, has been incorporated in several forms (acetate, diacetate, gluconate) to increase the antibacterial activity of dental composites and adhesives against cariogenic bacteria (Jedrychowski *et al.* 1983; Leung *et al.* 2005; Ionescu *et al.* 2013). Unfortunately, all forms of chlorhexidine used resulted in a porous surface (Anusavice *et al.* 2006), drop in the mechanical properties of the materials (Jedrychowski *et al.* 1983; Slot *et al.* 2011), increased water sorption (Deligeorgi *et al.* 2001) and short-term antibacterial effectiveness (Wilson and Wilson 1993). This could be attributed to chlorhexidine immiscibility with other dental monomers and associated difficulty in release rate control that make this approach not appropriate for dental applications (Zhang *et al.* 2014).

Nano-scale metal particles such as silver and zinc have been also used as bactericidal and bacteriostatic agents to impart antimicrobial properties to dental materials (Hernández-Sierra *et al.* 2008; Hojati *et al.* 2013).The antibacterial efficacy of metals is directly dependent on the total contact surface area. It has been claimed that among metallic agents, silver nanoparticles are the most effective agent for inhibiting the growth of *S. mutans* (Hernández-Sierra *et al.* 2008). Silver ions have been shown to inactivate enzymes and hinder DNA replication in bacteria (Chatzistavrou *et al.* 2014). However, poor colour stability is the major problem of silver containing restorative dental materials.

1.12.1.2 Immobilisation of antibacterial agents

Another approach to impart antibacterial activity into resin-based dental composites and adhesives is through the immobilisation of antibacterial components in the material. In this approach, the immobilised agent does not leach out from the material but inhibit via contact with the advantage of long-lasting antibacterial effects (Imazato 2003).

Quaternary ammonium compounds are effective antibacterial agents frequently used in oral hygiene products (Imazato *et al.* 2006). Several derivatives, which are ionic mono

and di-methacrylate monomers containing quaternary ammonium groups have been introduced to dimethacrylate-based dental materials demonstrating significant bactericidal effects against *S. mutans* and other oral bacteria (Farrugia and Camilleri 2015). Methacryloyloxydodecyl pyridinium bromide (MDPB) monomer, which is a combination of quaternary ammonium antibacterial agent and a methacryloyl group, has the ability to copolymerise with other monomers after curing of the composite and immobilize the antibacterial agents in polymer backbone to confer the polymer with long-term antibacterial effectiveness against *S. mutans* (Imazato *et al.* 1998; Imazato *et al.* 2012). The addition of this monomer into a self-etching adhesive system, present in Clearfil Protect Bond, increase its antibacterial effect against different oral bacteria (Korkmaz *et al.* 2008; Brambilla *et al.* 2013). However, it is thought that the acidity of self-etching primers containing MDP monomer is the main factor in providing their antibacterial effects.

Other monomers such as methacryloxylethyl cetyl dimethyl ammonium chloride (DMAE-CB) and Dimethyl aminododecyl methacrylate (DMAHDM) have also been added to dental bonding agents (Cheng *et al.* 2013) and composites (Zhang *et al.* 2015). It was found that dental adhesives containing these monomers can influence the biofilm formation and *S. mutans'* glucosyltransferases gene expression (Li *et al.* 2009). However, the immiscibility of some quaternary ammonium based monomers (Antonucci *et al.* 2012), cause deterioration in mechanical properties at high levels and the unwanted release are the main drawbacks of using these monomers (Ebi *et al.* 2001).

1.12.2 Natural plant-derived antimicrobial compounds

The use of compounds extracted from plants as antibacterial agents has recently become of great interest owing to the development of microbial resistance to the available therapeutic agents (Das *et al.* 2010). These plant secondary metabolites have potential in medical procedures and applications in the cosmetic, food and pharmaceutical industries.

Among these compounds, eugenol (4-allyl-2-methoxyphenol), the main constituent of clove oil from *Eugenia caryophyllata*, is an organic phenol with antipyretic, analgesic, anti-inflammatory, and anaesthetic effects (Kamatou *et al.* 2012). Eugenol exhibits antibacterial (Burt 2004), antifungal (Ahmad *et al.* 2010), antioxidant (Fujisawa and Kadoma 1997) and potential anticancer properties (Manikandan *et al.* 2010) demonstrated in pharmacological studies. Eugenol is also reported to exert inhibitory effects on MMPs via inactivation of ERK proteins chain in the cell (Kong *et al.* 2014).

The antibacterial activity and mechanism of action of eugenol and other essential oil products have been extensively studied (Kamatou *et al.* 2012; Kong *et al.* 2014). Eugenol exhibits potent activity against *Candida albicans* biofilms *in vitro* with low cytotoxicity (He *et al.* 2007; Ahmad *et al.* 2010). Several other studies have confirmed the antibacterial activity of eugenol against various pathogens such as *E. coli, B. cereus*, *Helicobacter pylori*, *S. aureus*, *S. epidermidis*, *Streptococcus pneumoniae, S. pyogenes* (Laekeman *et al.* 1990; Van Zyl *et al.* 2006) and *E. faecalis* (Dorman and Deans 2000) amongst numerous others. The results of recent studies revealed that eugenol at a sub-minimum inhibitory concentration (sub-MIC) can effectively suppress virulence genes of *S. mutans* (Adil *et al.* 2014) and has a strong inhibitory effect on their ability to produce adhesive glucan by glucosyltransferase (GTF) enzymes (Li *et al.* 2012; Xu *et al.* 2013) suggesting its therapeutic potential against oral biofilm and dental caries.

Regarding the mechanism of action, eugenol hydrophobicity is an important factor affecting its antimicrobial activity. The hydrophobic character of eugenol can separate the lipids of the cell membrane and mitochondria of bacteria and change its structure to increase penetrability of the cell membrane. Eugenol also has the ability to block the proton-motive force, electron stream, and active transport, and cause coagulation of cell contents (Kong *et al.* 2014). Another school of thought suggests that the site and the number of the hydroxyl group in phenolic compounds including eugenol is related to their antibacterial mechanism through more nonspecific interactions with the proteins and enzyme inhibition by the oxidised compounds (Burt 2004). A previous study revealed

that eugenol induced cell lysis through leakage of protein and lipid contents damaging the cell membrane of both Gram-negative and Gram-positive treated bacteria (Oyedemi *et al.* 2009).

Eugenol is widely accepted in many fields because of its minimal side effects, low toxicity and the broad range of pharmacological and biological activities. Apart from being used as a flavouring agent for foods, it has a long history of use in dentistry (Markowitz *et al.* 1992). Eugenol is commonly used in combination with zinc oxide (ZOE) as surgical dressings, temporary fillings, pulp capping agent, cement (Markowitz *et al.* 1992) and root canal sealer (Lai *et al.* 2001). Low concentrations of eugenol exert anti-inflammatory and local anaesthetic effects, relieving pain in irritated or diseased dental pulp and facilitate healing. On the other hand, high eugenol concentrations produce some adverse effects *in vivo*. Direct application of eugenol may result in extensive tissue damage of pulp tissue and induce inflammatory reactions over the oral mucous membrane.

One problem of eugenol-containing materials is the fact that they interfere with the polymerisation reaction of acrylic resins due to residual free eugenol (Hotz *et al.* 1992; Fujisawa and Kadoma 1997). This can deteriorate the physical and mechanical properties of the adjacent resin-based composite materials that cure mainly by free-radical polymerisation.

Numerous efforts have been performed in term of modifying the chemical structure of eugenol to improve its properties or to obtain additional pharmacological activities (Costa *et al.* 1994). A previous work by Luis *et al.* (2006) reported the ability of acrylic functionalisation of eugenol and synthesis of new eugenol derivatives that can participate in polymerisation reactions without the inhibitory effect characteristic of the phenol derivatives. One of these derivatives is eugenyl methacrylate (EgMA), a low molecular weight monomer with a polymerisable methacrylic group that allows the monomer to participate in free radical polymerisation reactions whilst maintaining the antibacterial activity of its natural precursor against different Gram-negative and Gram-positive

bacterial species (Rojo *et al.* 2008a). The new derivative could be incorporated into methacrylate based restorative materials, imparting bactericidal effects of eugenol.

1.12.3 Assessment of antibacterial activity of dental materials

Agar diffusion test (ADT), which is based on measuring water-soluble components released from the bulk of the materials, has been commonly used as a standard assay for assessing the antibacterial activity of dental restorative materials. However, the main disadvantage of the ADT is the lack of ability to distinguish between bacteriostatic and bactericidal properties of test materials. Because of the qualitative nature of the test, it fails to provide any information about the viability of the test bacteria (Tobias 1988).

The main methods described for testing are direct contact test, determination of colony forming units (CFU), a broth culture test, Methyl thiazolytetrazolium (MTT) assays, SEM and live/dead cell viability assay (Farrugia and Camilleri 2015).

In direct contact test, microorganisms are allowed, under controlled conditions, to contact the tested material directly, in order to study the kinetics of bacterial growth. It can test the effects of this contact on microbial viability, regardless the solubility and diffusibility of antimicrobial components (Beyth *et al.* 2007).

Counting the number of colonies (CFU) in agar plates is the most widely used method to quantify the viable bacteria recovered from biofilm growth or planktonic environments formed on the surface of restorative materials (Hojati *et al.* 2013). Recently, Live/Dead BacLight®bacterial staining along with the CLSM technique has been increasingly used in biofilm related research providing data on the amounts of viable bacteria (Pihl *et al.* 2010; Niazi *et al.* 2014; Niazi *et al.* 2015). The CLSM technique can be utilised to complement and verify the established microbiological cultural techniques for investigating the antimicrobial efficacy of different materials against biofilm bacteria.

Summary

To date, although many post and core materials have been used for restoration of structurally compromised ETT, there is no ideal and fully approved system. The multiple requirements of these materials and the complexity of treatment procedure necessitate the need to develop and assess new restorative materials meeting all criteria and replacing the available systems which have some limitations. Although the commercially available translucent or tooth-coloured fibre posts combined with resin composites and adhesives systems have gained popularity and acceptance among clinicians, data from both *in vivo* and *in vitro* studies report a relatively higher failure rate for ETT with fibre post restorations. Post debonding, recurrence of endodontic lesions and secondary caries are the frequent failure modes identified.

Recent trends in restorative techniques indicate that the newly developed materials should not aim to create the stiffest restoration, but rather to create a biomimetic material similar to their natural counterparts in terms of biology, function, mechanics, and aesthetics. Another important clinical issue that has not been fully investigated is post retrievability in case retreatment is required.

There have also been several attempts to incorporate polymerisable antimicrobial compounds into the dental composites and adhesive materials, which is expected to lower the risk of reinfection and secondary caries, enhancing the longevity of restorations. However, bacteria that persist within or invade the root canal system following RCT are primarily organised as biofilms with enhanced pathogenic potential. These communities of bacteria have the capability to adapt to the harsh environment of root canal-filled teeth and display an increased tolerance to antimicrobial agents. Therefore, the selected antibacterial agent should provide a high potency to tackle the intraradicular biofilm and reduce the risk of treatment failure.

Research aims

The overall aim is to develop a post and post-core system tailored to suit the effective treatment of endodontically treated teeth.

Objectives of this project:

1. Development and characterisation of thermoplastic polymer based composites from LDPE and HA tailored to function as intracanal post materials using a melt extrusion process.

2. Optimisation of surface treatment of high fatigue-resistant Kevlar 49® fibres and evaluation of the feasibility of using treated Kevlar fibre in combination with the semi-IPN based matrix as new FRC post material through extensive characterisation and comparing the results with those of a commercial glass fibre reinforced semi-IPNs post material (everStick®POST).

3. Imparting an antimicrobial property into experimental resin composites core, designed for intracanal post placement by incorporation of eugenyl methacrylate (EgMA) antibacterial monomer.

4. Modification of two commercial dental adhesive systems (self-etch and total-etch) via the inclusion of the EgMA for their application in post-endodontic restorations to reduce the risk of reinfection and secondary caries.

5. Evaluate the influence of monomer addition on curing kinetics, behaviour, physical and mechanical properties of the newly developed composites and modified adhesives materials.

6. Qualitative and quantitative assessment of the bonding ability, cytotoxicity and antibacterial activity of the experimental resin composites and modified adhesives.

Chapter 2 Development and characterisation of low density polyethylene-hydroxyapatite composite post material by melt extrusion process

This chapter includes the fabrication and characterisation of PE-HA composite post materials (section 2.1, Almaroof *et al.* 2015) and further supplementary information regarding the silanation and optimisation of HA filler within the composites and the removal protocol of PE-HA experimental post (section 2.2).

2.1 New functional and aesthetic composite materials used as an alternative to traditional post materials for the restoration of endodontically treated teeth

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Authors' contribution

I was responsible for the experimental work, data analysis and designing of the paper. I also produced figures and tables and wrote a draft of the manuscript, which was edited and reviewed by the co-authors. Dr Deb supervised the development of the work.

Abstract

Objectives: To tailor composites of polyethylene-hydroxyapatite to function as a new intracanal post for the restoration of endodontically treated teeth (ETT).

Methods: Silanated hydroxyapatite (HA) and zirconium dioxide (ZrO_2) filled low-density polyethylene (LDPE) composites were fabricated by a melt extrusion process and characterised using infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA). The flexural strength and modulus were determined in dry state and post ageing in simulated body fluid and fractured surfaces analysed by SEM. The water uptake and radiographic appearance of the experimental

composites were also measured and compared with a commercially known endodontic fibre post. Data were submitted to one-way analysis of variance (ANOVA) and post-hoc Tukey multiple comparison tests at a level of significance $P<0.05$.

Results: The LDPE/HA composites were structurally flexible and the HA content had a significant effect on the flexural strength and modulus. A univariate analysis of variance showed no significant differences in modulus and strength ($P>0.05$) post accelerated ageing in simulated body fluid with very low water uptake. The melting point of the LDPE/HA composites ranged between 135 and 136 °C, which would facilitate removal in case of retreatment using conventional dental heating devices. The inclusion of HA reduced the damping thereby enhancing dimensional stability, whilst the addition of zirconia yielded a semi-translucent material that was sufficiently radiopaque, comparable to commercial posts, thus yielding aesthetic materials.

Conclusions: Innovative materials for restoration of ETT were developed; offering considerable benefits over the currently available material in terms of biomechanical and thermal properties.

Clinical significance: This study provided a new option for the development of a new intracanal post made up of functional and aesthetic composites.

2.1.1 Introduction

Endodontically treated teeth are more susceptible to fracture than vital teeth mainly because of the reduction of tooth structure (Zicari *et al.* 2013b), changes in the chemical composition of dentine due to loss of water and collagen alteration might also play a part in the reduced resistance to fracture of ETT (Rivera and Yamauchi 1993). A post-core restoration is usually required if a large amount of tooth structure is lost to allow retention of the final restoration (Schwartz and Robbins 2004).

Metallic cast and prefabricated posts have been used for decades; the main disadvantages of these posts are their high modulus of elasticity, which may be associated with catastrophic fractures of the root (Santos-Filho *et al.* 2008; Gómez-Polo *et al.* 2010) and unpleasant metallic colour, particularly when placed underneath all-ceramic anterior crowns. More recently, metallic posts are being replaced by metal–free post systems such as zirconia and fibre reinforced composite (FRC) posts to improve both mechanical and aesthetic properties. However, the high elastic modulus and rigidity of the zirconia post (Asmussen *et al.* 1999) may also cause root fractures, which are also very difficult to remove when a failure occurs.

FRC posts have the potential to lower the risk of vertical root fracture due to their lower modulus of elasticity, whilst providing adequate aesthetics (Plotino *et al.* 2007a), however the relatively high rigidity of FRC posts has also been questioned (Raygot *et al.* 2001). In more complex cases where a significant amount of coronal and radicular tooth structure is lost, such as in flared root canals with thin dentine walls, filling the root canal space with a stiff material may increase the chance of root fracture (Sirimai *et al.* 1999). In such cases, the prefabricated fibre posts may not ideally fit the root canal space and require more luting cement that may lead to inefficient bonding (Boschian Pest *et al.* 2002). It has been suggested that accessory glass fibre posts can be used in root canals with thin-walled roots (Bonfante *et al.* 2007), however, the presence of accessory posts although able to minimise the cement layer, do not improve fracture resistance (Latempa

et al. 2015). Additionally, most currently available FRC posts essentially consist of a thermoset matrix with a high degree of conversion and a highly cross-linked network that binds the fibres in the composite structure, which makes their removal difficult in the event of a nonsurgical endodontic retreatment (Ruddle 2004; Mannocci and Cowie 2014). Therefore, there is a need to develop new restorative materials with desirable properties as alternatives to the currently available systems.

Hydroxyapatite-particle-reinforced polyethylene composite was introduced in the early 1980s as a bone analogue (Bonfield *et al.* 1981) and has been clinically used for many biomedical devices such as middle-ear implants (Tanner 2010). The flexibility and biocompatibility make this composite a good candidate in replacing the conventional implant materials (metals and ceramics) which have a higher modulus that exceeds that of the surrounding host tissue and results in stress shielding and causes prosthesis failure. The rationale of selecting polyethylene and hydroxyapatite (HA) suited for posts in endodontic restorations stemmed from the excellent established cytocompatibility, the mineral component similar to that of the mineral content of dentine and polyethylene as a bio-inert matrix which can act as an analogue to collagen (Lim *et al.* 2006). Recently, promising materials from a low molecular weight low density polyethylene (LDPE) with HA filler have been reported and their efficacy as new obturating materials for endodontic applications has been described (Alhashimi *et al.* 2013). In the present study, a relatively high molecular weight, low density polyethylene was selected as a matrix which was reinforced with silanated HA and radiopacifying filler to develop new composites tailored to function as an endodontic restorative material. The null hypothesis is that PE-HA composites are not appropriate as post materials for restoration of structurally compromised ETT.

2.1.2 Materials and methods

2.1.2.1 Materials

The properties of polyethylene used for this study are presented in Table 2.1-1. Hydroxyapatite (HA) powder with a mean particle size diameter of 3-5 µm was procured from Plasma Biotal Ltd. (Derbyshire, UK). Zirconium oxide (ZrO_2) (Fisher, UK) with a mean particle size diameter of 18 µm, silane coupling agent A-174 (3 Trimethoxysilyl propylmethacrylate), (Merck, Frankfurt, Germany) were used in this study. The size of the filler particles were characterised using a CILAS 1180 laser diffraction particle analyser (Cilas, Orleans; France). The hydrolytic stability and radiographic appearance of the experimental composites were compared with a commonly used fibre reinforced post material, Rely X (3M ESPE, USA) tested under identical conditions of the experimental composites and used as a reference only.

Table 2.1-1 Properties of polyethylene

PE type	Manufacturer	Form/particle size	Molecular weight	Density	Melting point
Low density PE	Goodfellow/England	Powder/1000 µm	400,000 g/mol.	0.918 g/cm³	140 °C

2.1.2.2 Silanation of HA and ZrO_2 fillers

In order to enhance the interfacial adhesion between the polyethylene matrix and the inorganic filler, hydroxyapatite and zirconium oxide particles were subjected to a wet silanation method using A-174 in a 70/30 mix of acetone and distilled water (Deb *et al.* 1996). The silanated HA and ZrO_2 particles were characterised using FTIR to confirm the silanation.

2.1.2.3 Fabrication of composites

Two sets of composites were prepared with low density PE as the matrix based on range finding experiments for ease of processability. The composition is shown in Table 2.1-2.

Table 2.1-2 Composition of the experimental composites (wt.%)

Composite	LDPE	Silanated HA	Silanated ZrO_2
LDPE Pure	**100**	**0**	**0**
LDPEHA40ZR10	**50**	**40**	**10**
LDPEHA30ZR20	**50**	**30**	**20**

Polyethylene (PE) powder and silanated HA and ZrO_2 particles were mixed in the above proportions. The mixture was then fed continuously into a barrel of single screw extruder (12 mm single screw, Rondol Technology) at a screw speed of 25 rpm. The processing temperature was set between 180–190 °C, which was the most suitable for LDPE processing (Rosa *et al.* 2007). The materials were then extruded through a die, drawn down to different diameters and tapers (Fig. 2.1-1a), cooled to room temperature and stored in a desiccator. Additionally, a custom post was successfully made from these materials by investing and flasking a wax post and core pattern (Fig. 2.1-1b).

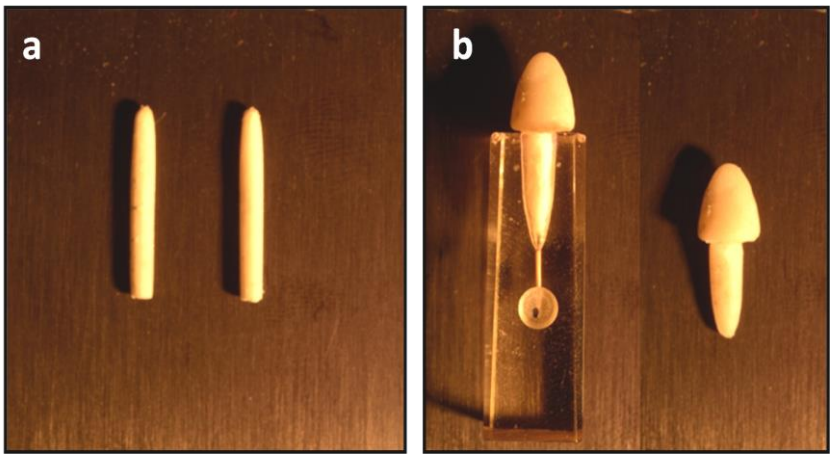


Figure 2.1-1 (a) Extruded fibres in different diameters and tapers obtained from the melt-extrusion process of LDPEHA40ZR10. (b) Custom post and core fit on simulated flared canal prepared in an acrylic block obtained by flasking of LDPEHA40ZR10

2.1.2.4 Spectral analysis of the composites by FTIR

Fourier transform infrared (FTIR) spectroscopy (ATR accessory, Spectrum one, Perkin Elmer, Waltham, MA, USA) was used to confirm the presence of silane coupling agent and characterise the chemical composition of the fabricated composites.

2.1.2.5 Thermal analysis

Differential scanning calorimetry (DSC) was carried out using a Perkin Elmer equipment (Waltham, MA, USA) to measure the melting and crystallisation temperature of the composites. Samples of about 10 mg were heated to 200 °C at the rate of 20 °C/min in an inert atmosphere (N_2 atmosphere). Two heating/cooling cycles were carried out for each composite group; the data was calculated from the second heating cycle to avoid the effect of impurities on the thermal history during processing which may influence the thermal analysis data.

Dynamic mechanical analysis (DMA) was performed using a DMA7 (Perkin-Elmer, USA) in bending mode to measure the storage modulus (E'), loss modulus (E''), damping (tan δ) and glass-transition temperature (Tg) of the composites. A frequency of 1 Hz was applied (approximately average chewing rate) and a temperature range between -100 to 50 °C (heating rate of 2 °C/min) was selected to cover glass-transition temperature (Tg) of the matrix and oral temperature. Rectangular bar specimens of dimension (40 x 2 x 2 mm) were fabricated by special heated mould and used for DMA test with n=3.

2.1.2.6 Mechanical properties

The static mechanical properties of composites were determined dry and after 3 months storage in simulated body fluid (SBF) using a universal testing machine (Instron, M 5569A). A three-point bending test was carried out according to ISO 10477 standard at a cross-head speed of 1 mm/min and span length fixed at 20 mm. Six specimens (2 mm x 2 mm x 25 mm) of each composite were fabricated by melting the materials in a standardised stainless steel heated mould. Flexural strength and flexural modulus were measured and load–deflection curves recorded with PC-software.

2.1.2.2.7 Fracture surface examination

The fractured surfaces of the LDPEHA40ZR10 and LDPEHA30ZR20 composites from the dry samples were gold coated and examined using Scanning Electron Microscopy (SEM, Hitachi High Technologies, S-3500N) at an accelerating voltage of 10 KeV and magnifications 1000x and 5000x.

2.1.2.8 Water uptake

Five cylindrical specimens (2 mm x 20 mm) of each test materials were compared with five Rely X posts of the same dimensions. All specimens were weighed thrice using calibrated electronic microbalance (Mettler; Toledo; Switzerland); the average reading was recorded to the nearest 0.0001g. The specimens of each group were kept in individual containers in 10 ml deionized distilled water at 37 °C. All specimens were periodically weighed, prior to weighing; the specimens were taken out of water, gently dried with filter paper and left undisturbed for 1 minute in order to allow stabilisation of each specimen. The weight measurements were taken every day for the first week, then weekly for the next 3 months and finally once a month up to 6 months. The percentage weight changes were calculated using the following formula:

$$\text{Weight change} = (W_t - W_0) / W_0 \times 100$$

where W_t is the weight at time and W_0 is the original weight of the specimen.

2.1.2.9 Radiographic assessment

The radiopacity of the composite fibres was compared with the radiopacity of Rely X post under two different conditions (outside and inside the root canal) using digital dental radiographic images (storage phosphor plate, Digora® Optime; Soredex, Tuusula, Finland) obtained with a dental X-ray unit (Heliodent; Sirona, Bensheim, Germany) operating at 70 kV, 8 mA, and 0.2 seconds. The post's image outside the root was obtained simply by placing it on the surface of the film, whilst for the latter, a dry mandible containing a crown sectioned tooth was used, the canal of the root was filled with gutta-

percha and two thirds of the filling was removed to receive a post (2 mm in diameter). The dry mandible was seated in a medium body silicone putty mould (Dentsply, Germany) and a 1 cm acrylic plate (Plexiglas; Evonik, Essen, Germany) was placed between the X-ray source and the mandible to simulate the presence of the soft tissues in the clinical situation. The film was fixed in position and aligned with the X-ray source using paralleling technique with a focus to film distance of 30 cm.

2.1.2.10 Statistical analysis

The results were analysed using one-way analysis of variance (ANOVA) followed by Tukey multiple comparison post-hoc tests at a level of significance $P<0.05$. SPSS software version 20 was used to analyse the data.

2.1.3 Results

2.1.3.1 Spectral analysis of the composites by FTIR

The FTIR spectrum of the silanised hydroxyapatite showed absorption bands at 1720, 1637, 1460 and 1300 cm^{-1} that were attributed to C=O, C=C, C-C and Si-O, respectively, indicating the availability of the coupling agent on the surface of hydroxyapatite (Fig. 2.1-2A). The FTIR spectra of LDPEHA40ZR10 and LDPEHA30ZR20 were very similar to each other as expected; however, the intensity of the phosphate peaks varied because of the compositional changes as shown in Fig. 2.1-2B.

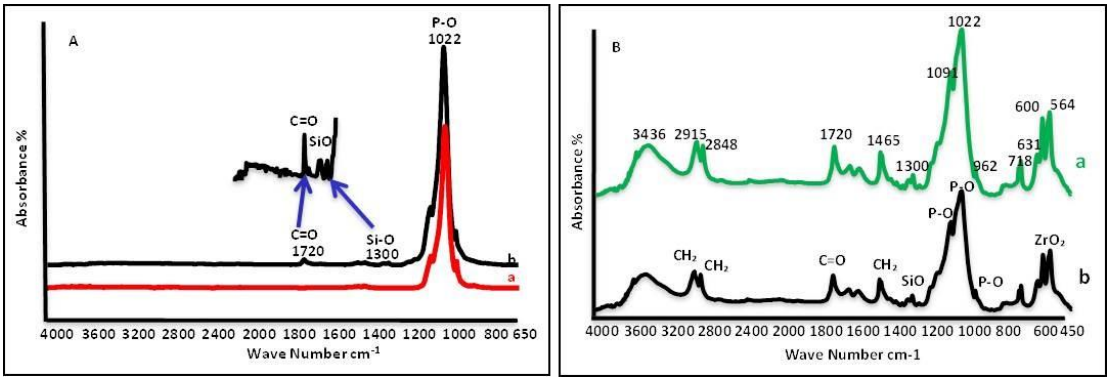


Figure 2.1-2 FTIR spectra of (A) non-silanated HA (a) and silanated HA (b). (B) Experimental composites LDPEHA40ZR10 (a) and LDPEHA30ZR20 (b)

The strong absorption peaks at 2915, 2848, and 1465 cm^{-1} confirm the existence of methylene ($-CH_2-$) group arising from polyethylene segments, the absorption bands at 962, 1022 and 1091 cm^{-1} were assigned to the phosphate groups, P-O present in the hydroxyapatite. A broad vibration band in the region 500-600 cm^{-1} reflects the presence of zirconium oxide, whilst the broad band at 3436 cm^{-1} is associated with the OH stretching vibrations of water molecules on the ZrO_2 surface. The absorption band at 1720 cm^{-1} (C=O) indicated the presence of silane coupling agent in the composites.

2.1.3.2 Thermal analysis

The thermal properties of the experimental composites are summarised in Table 2.1-3. The melting temperature (Tm) of the composites ranged between 135 and 140 °C, which remained nearly unaffected with the inclusion of various filler content. The crystallisation temperatures (Tc) of the composites remained unaffected, which indicated the thermal properties of the LDPE matrix was retained, hence the second phase interference and processing technique did not cause any macro-scale changes.

Table 2.1-3 Thermal properties and dynamic mechanical analysis of the composites (mean ± S.D., n=3)

	DSC		DMA			
Composite	**Tm (^{0}C)**	**Tc (^{0}C)**	***T*g (^{0}C)**	**E′(GPa) at 37 ^{0}C**	**E″(GPa) at 37 ^{0}C**	**tanδ (x10^3) at 37 ^{0}C**
LDPE Pure	**140.2 ± 1.0**	**115.0 ± 0.5**	**-70.4 ± 1.2**	**6.50 ± 0.5**	**0.99 ± 0.1**	**152 ± 2.6**
LDPEHA40ZR10	**135.2 ± 0.8**	**116.4 ± 0.6**	**- 60.8 ± 1.3**	**14.16 ± 0.2**	**1.81 ± 0.3**	**128 ± 2.1**
LDPEHA30ZR20	**136.1 ± 1.2**	**116.5 ± 0.8**	**- 63.3 ± 0.6**	**11.87 ± 0.3**	**1.63 ± 0.03**	**137 ± 2.0**

The mean values of storage modulus, loss modulus and damping factor (tan δ) at 37 °C of the experimental composites are shown in Table 2.1-3. The storage modulus values clearly indicated the inclusion of hydroxyapatite as a filler within pure LDPE had a stiffening effect, whilst the Tan δ values in contrast decreased with increasing HA volume

fraction. However, LDPEHA30ZR20 composites exhibited lower modulus and higher damping than LDPEHA40ZR10 composite indicating that HA volume fraction had a more pronounced effect on stiffness than the radiopacifier zirconia. The peak in the tan *δ* versus temperature curves corresponds to the drops in the storage modulus curves and represents the *T*g values for the composites (Fig. 2.1-3). The *T*g peaks of the filled composites shifted toward higher temperatures and their values were significantly higher (*P*<0.001) in comparison to pure LDPE.

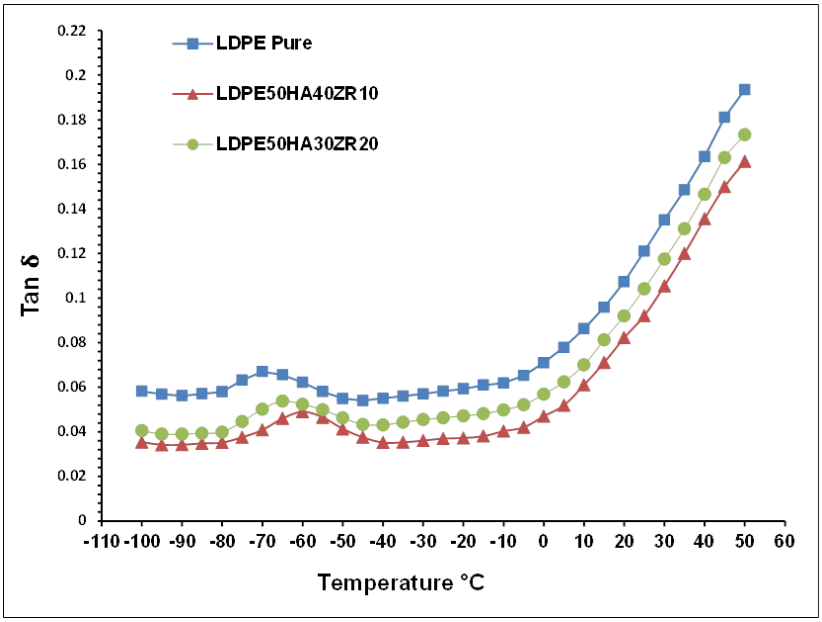


Figure 2.1-3 Tan *δ* versus temperature for pure LDPE and experimental composites

2.1.3.3 Mechanical properties

The flexural strength (a) and modulus (b) of the composites under dry conditions and after 3 months storage in SBF at 37 °C are shown in Fig. 2.1-4. The flexural strength and modulus of LDPESHA40ZR10 and LDPESHA30ZR20 composites were significantly higher (*P*<0.0001) than that of pure LDPE under both conditions. The hydroxyapatite weight fraction had a significant effect on the flexural strength with the composite containing 40% HA weight fraction, exhibiting a higher flexural strength (*P*<0.05); however, it did not result in a significant difference in the flexural modulus. The univariate analysis of variance showed no significant differences in modulus and strength (*P*>0.05) after 3 months storage in SBF in all groups.

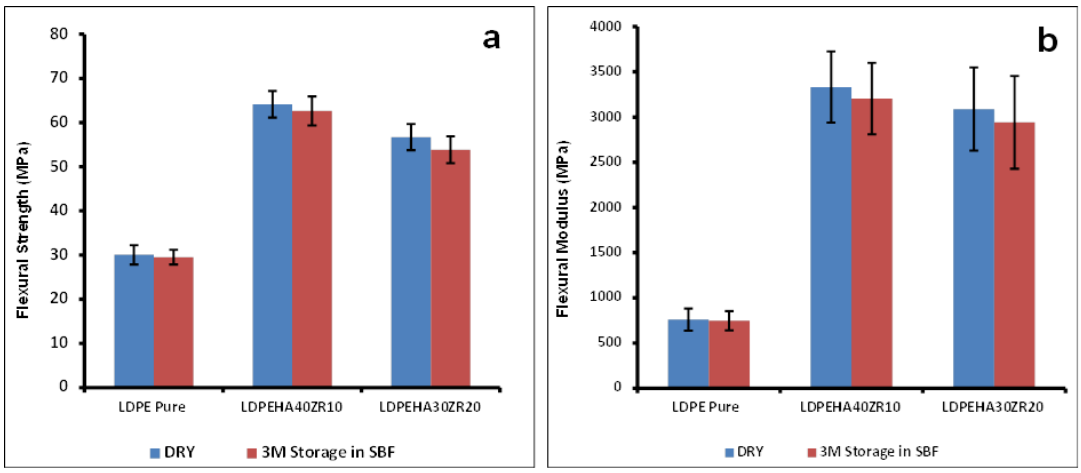


Figure 2.1-4 (a) Flexural strength and (b) Flexural modulus of the experimental composites

2.1.3.4 Fracture surface examination

Representative SEM images of the fractured surfaces obtained from the flexural test specimens of the two filled composites are shown in Fig. 2.1-5. The images indicate that both composites exhibited ductile mode of failure.

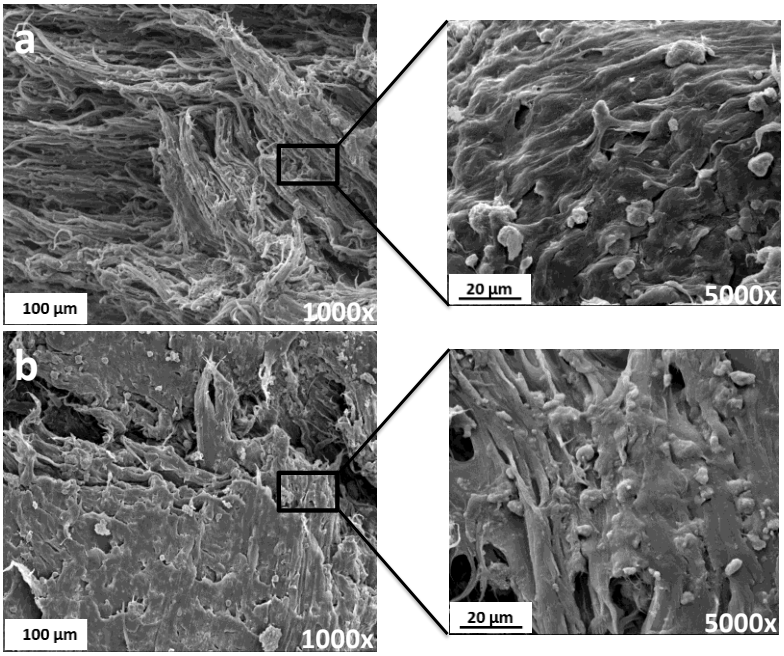


Figure 2.1-5 Morphology as observed by scanning electron microscopy (SEM) of three-point bending fracture surfaces of dry experimental composites at different magnification. (a) LDPEHA40ZR10 and (b) LDPEHA30ZR20

2.1.3.5 Water uptake

The gravimetric changes over time on immersion of the composites are presented in Fig. 2.1-6. The rate of water sorption in the early stages was higher in the composites in

comparison to the hydrophobic LDPE as expected. All the experimental composites equilibrated within two weeks with a very low net water uptake, which was comparable to the commercial materials. However, Rely X post, a commercial material showed a greater uptake and did not equilibrate and continued to increase with time over total immersion period.

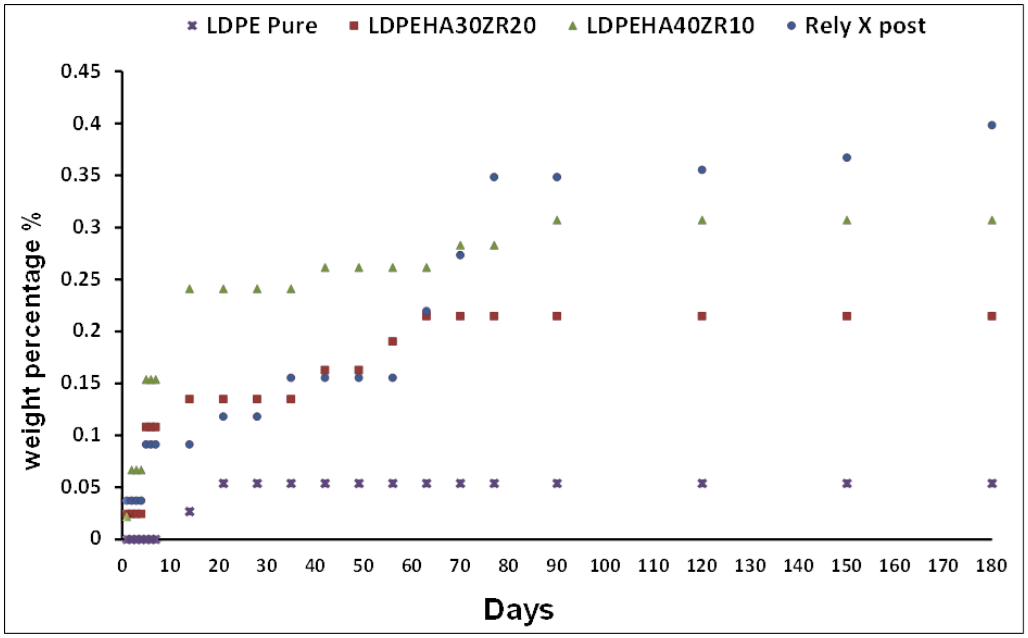


Figure 2.1-6 Mean percentage changes in weight over 180 days immersion of studied materials

2.1.3.6 Radiographic assessment

The radiographic images of the posts outside the root (Fig.2.1-7A) and inside the root canal (Fig. 2.1-7B-D) showed that the experimental composites appeared to be satisfactorily radiopaque and homogenous. However, radiopacity of composite containing 20% ZrO_2 was superior to that containing 10% ZrO_2 and was comparable with the radiopacity of commercial Rely X post.

Figure 2.1-7 Digital radiographic images (A) Posts outside the root: (1) LDPEHA40ZR10 post, (2) LDPEHA30ZR20 post and (3) Rely X post. (B) LDPEHA40ZR10 post within the root canal. (C) LDPEHA30ZR20 post within the root canal. (D) Rely X post within the root canal

2.1.4 Discussion

The rigidity of the restorative material is regarded as one of the most influential parameters affecting the mechanical behaviour of ETT (Santos *et al.* 2010). Composites based on thermoplastic polymers are generally less stiff and their mechanical properties can be tailored according to the particular application by varying the proportions and properties of the matrix and the filler. Polyethylene is a useful thermoplastic, biocompatible polymer, available in different densities and molecular weights. It has been increasingly used in current medical devices because of its high Young's modulus, abrasion, and wear resistance (Whitehouse *et al.* 1999). In this study, a relatively high molecular weight low-density polyethylene was selected to achieve an adequate strength. Moreover, the flow property and processability of LDPE allows the incorporation of various types of fillers possible through melt mixing.

Hydroxyapatite is a natural component of dentine with established biocompatibility to suit biomedical applications in addition to intrinsic radiopacity and ideal hardness (Zhang and Darvell 2012). The addition of 30% and 40% weight hydroxyapatite was based on a pilot study that showed optimum handling and mechanical properties, consistent with the findings of earlier studies that reported brittle behaviour and processing difficulties of the composites when the HA concentration was increased (Tanner 2010). The net filler content was maintained at 50% by weight in both the experimental composites with the addition of 10% and 20% wt. of ZrO_2 to impart required radiopacity. Furthermore, ZrO_2 was used because of its high strength, fracture toughness and biocompatibility (Silva *et al.* 2001).

The inorganic fillers (zirconia and hydroxyapatite) and organic matrix (PE) are essentially incompatible; hence surface treatment of the particles was carried out prior to formulation of the composites to enhance the interfacial bonding, improve the mechanical properties (Deb *et al.* 1996) and reduce the water sorption of the composites (Demir *et al.* 2006).

The FTIR spectra of the composites showed characteristic absorption bands arising from methylene groups, which confirmed the presence of the polyethylene matrix with no peaks arising from any oxidative degradation and the peaks at 1022 cm^{-1} and 600 cm^{-1} confirmed the presence of HA and ZrO_2 respectively. This is in agreement with most research reports (Santhoskumar *et al.* 2010; Wu *et al.* 2014).

DSC thermograms of neat LDPE showed a melting temperature of 140 °C and the addition of silanated fillers resulted in slight shift in melting temperature to lower value with absence of any tangible changes in the crystallisation temperatures. This finding indicates the thermal stability of the resultant composites, which is in agreement with similar findings reported for HDPE/HA bio-composites (Jaggi *et al.* 2012).

Post removal can be less or more difficult depending on the type of material from which it is made. Many techniques and instruments have been used for the removal of posts, ultrasonic vibration being the most common and safe. However, heat generation increases the temperature of the external root surface above the periodontal ligament's threshold (47 °C) (Ettrich *et al.* 2007), which is one of the limitation of such a device (Scotti *et al.* 2013), especially when applied over longer periods. The removal of all metallic posts is often time-consuming and is associated with the risk of root perforation (Gesi *et al.* 2003). Fibre post removal can be accomplished by drilling (trophing) through the middle of the post with the possibility of root perforation and tooth structure loss (Mannocci and Cowie 2014). Nonsurgical retreatment of fibre posts cemented with adhesive systems is one of the challenges in endodontics (Plotino *et al.* 2007b), especially for tapered or large diameter canals in which more tension is needed to remove a wide post (Alfredo *et al.* 2004). The experimental polyethylene–hydroxyapatite composites developed in this study exhibited melting points that ranged between 135 and 136 °C, and thus have the ability to be plasticised and removed at a temperature between 85 to 100 °C using conventional dental heat sources (Alhashimi *et al.* 2012), this temperature is far below the ideal operating temperature of the devices usually used to convey heat into the root canal (200 °C) (Lipski 2005). This is clinically advantageous

and will allow the experimental post material to be retrieved from the canal easily and safely, whereas other post materials do not offer this option due to their much higher melting points.

Dynamic mechanical analysis for the HA filled composites and pure PE were performed to examine the effect of fillers on the thermo-mechanical properties of the composites. In this study composites with 40 wt % HA showed the highest storage modulus (14.16 ± 0.2 GPa at 37 °C) and highest *T*g value (- 60.8 ± 1.3 °C). This is attributed to the uniform dispersion of HA and zirconia particles in PE matrix. The addition of fillers limited the mobility of the amorphous phase in the polymer, hence reduced the damping of the composite and increased its dimensional stability, the degree of which depends upon HA content.

For long-term tooth survival, the restoration of root filled teeth should maintain function, aesthetics and protect the tooth against fracture (Taha *et al.* 2011). The experimental materials have an acceptable tooth-coloured appearance in comparison to metallic posts, thus can contribute to the optical properties of all-ceramic overlying restorations, and are structurally flexible. Endodontic restorative materials also should have a modulus of elasticity similar to that of dentine (18-20 GPa). A study on a range of endodontic post materials reported that the values of flexural modulus of the tested posts were from 2–6 times (FRC) to 4–10 times (metal) that of dentine and the flexural strength of FRC endodontic posts exceeded the yield strengths of metal posts (Stewardson *et al.* 2010b). The flexural properties of the experimental composites showed acceptable flexural strength, above 50 MPa (the minimum flexural strength stated by the ISO 10477) and flexural moduli ranged between 3.0 to 3.3 GPa. Despite the significantly lower strength values achieved for the resultant composite materials when compared with that of metal and some fibre posts found in literature, their performance may be considered favourable in a structurally weakened root with a wide flared canal as the prognosis of a long term successful restoration of the tooth is compromised, taking into consideration that the

mechanical properties of posts depend largely on the diameter of the post (Lambjerg-Hansen and Asmussen 1997).

SEM examination of the fracture surfaces obtained from the bending test revealed a uniform distribution of the fillers throughout the matrix and the shrinkage of LDPE around individual particles during thermal processing suggesting better compatibility and adhesion (Fig. 2.1-5). Moreover, the predominant failure mode was due to the matrix LDPE and showed ductile fracture behaviour for both filled composites.

The new composite materials showed a very low net water uptake between 0.2 and 0.3 wt.%, which was far below the clinically acceptable water-uptake values of composites (2-3 wt.%) (Domingo *et al.* 2003). A previous study has also shown that the addition of HA to 40 wt.% decreased water absorption of PE based composites (Zuo *et al.* 2007). Furthermore, these findings support the results obtained from testing the effect of wet ageing on the mechanical properties of the composites, which showed the retention of their flexural properties after 3 months ageing in SBF. Posts may be subject to saliva only if the breakdown of the adhesive interface occurs. However, the stability over three months in a completely immersed state, which is an extreme condition, indicates long term ability to survive in clinical environments. Water sorption can cause several physical and chemical changes in composite materials such as softening, plasticisation, oxidation and hydrolysis. Negative effects caused by water exposure in fibre posts have been reported due to the reduction in their physical properties as a result of hydrophilicity of the resinous matrix (Mannocci *et al.* 2001b; Stewardson *et al.* 2010a). The hydrophobic properties of PE and the hydrolytic stability of HA result in the lower water sorption of the experimental composites that offer a considerable advantage over FRC posts.

Radiopacity is an important property for endodontic posts selection, which allows their clinical detection. The chemical composition and radiopaque elements of fibre posts contribute to their radiographic appearance. The experimental composite with 20% of

zirconia exhibited a radiopacity comparable to that of the commercial fibre posts used as control and was easily distinguishable from dentine and gutta-percha.

The null hypothesis of the present study is rejected as the fabricated composites exhibit appropriate properties as potential new endodontic post materials with respect to biomechanical behaviour, hydrolytic stability and retrievability. Although further studies employing stress analysis and bonding are necessary for the clinical application of these materials, their suitability to restore the structurally compromised ETT appears to be a promising alternative to currently available post systems.

2.1.5 Conclusions

The experimental composites fabricated with established biocompatible materials showed potential as a post material for restoration of ETT. The composites exhibited lower water sorption, good aesthetics, sufficient radiopacity and a Tm that would enable easy retrieval from the root canal in case of a retreatment. The structural and thermal analysis indicated the stability of the novel composites. The newly developed materials showed a high degree of flexibility suggesting their potential application and a favourable stress distribution in a root canal with flared and thin dentine walls, potentially lowering the risk of fracture.

2.2 Supplementary information

2.2.1 Silanation effect and optimisation of HA filler contents of PE-HA composites (a pilot study)

Silane coupling agents are commonly used to enhance the interfacial adhesion between two dissimilar constituents of a composite that impacts thermal, physical and mechanical properties (Wang and Bonfield 2001). Coupling agents can improve the wetting and dispersion of the fillers in the polymer matrices (Labella *et al.* 1994; Wang *et al.* 2000) and have a general formula: X3SiRY.

X represents a hydrolysable group, Y is an organofunctional group. The organofunctional groups are chosen for reactivity or compatibility with the polymer, while the hydrolysable groups are merely intermediate in the formation of a bond with the filler or fibre surface. During the treatment of mineral surfaces with silane coupling agents, the alkoxy groups (RO–) hydrolyse in an aqueous environment producing hydroxyl groups (Fig. 2.2-1).

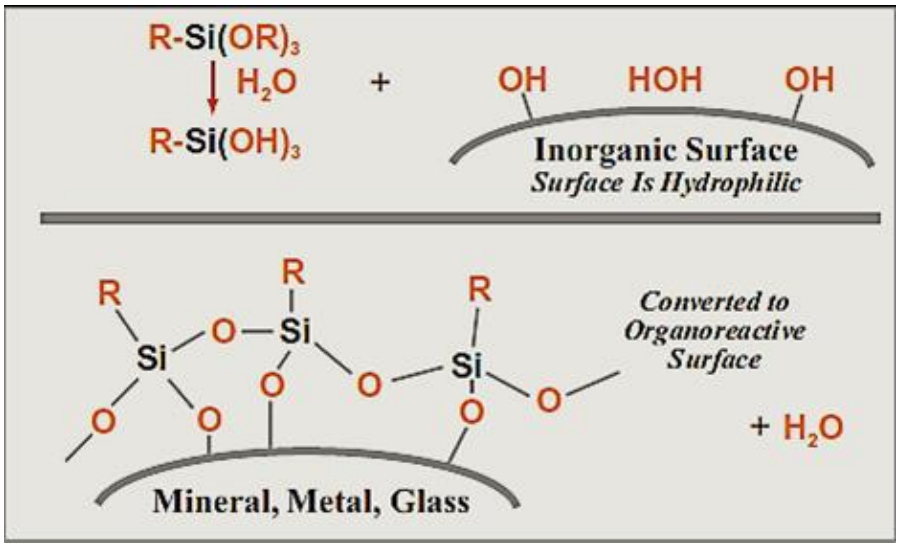


Figure 2.2-1 Treatment of a filler surface with an organosilane (adapted from www.Xiameter.com 2009)

The mechanical and thermal properties of silanated HA-PE composites were evaluated and the concentration of HA filler was also optimised.

2.2.1.1 Silanation of hydroxyapatite

HA particles were treated with silane coupling agent using a procedure described by Deb *et al*. (1996). 3 trimethoxysilyl propylmethacrylate (A-174) 12.5% by weight (with respect to hydroxyapatite) was mixed in a 70/30 acetone/water solution and hydroxyapatite particles were added to it and stirred with a magnetic stirrer at room temperature for 1 h and the temperature gradually raised to 100 °C whilst stirring and maintained for 1 h to obtain a slurry. Thereafter, the hydroxyapatite slurry was placed in an oven maintained at 125 °C for 2 h until the solvent had evaporated. The coated hydroxyapatite was washed with water, centrifuged and dried at 37 °C (Deb *et al.* 1996).

2.2.1.2 Processing of LDPE/HA composites fibres

Unfilled low-density polyethylene (LDPE) with 20, 40 and 50 percent by weight of silanated and unsilanated hydroxyapatite composites fibres were produced using a 12 mm single screw extruder (Rondol Technology, Staffordshire, UK) for comparison of the mechanical properties of the composites. The formulation and compositions of the composites used in this pilot study are summarised in Table 2.2-1.

The LDPE powder was mixed with the HA and then fed continuously into a barrel of single screw extruder at a screw speed of 25 rpm. The processing temperature was set to a 180–190 °C temperature range. Since the homogeneity of a composite is crucial, the feeding rate of the mix into the barrel was kept low and the barrel blocked off at the lower end to allow the loaded barrel to equilibrate at the set temperature for 2-3 min. Extrusion was then carried out through a 2 mm diameter die, cooled to room temperature and stored in a desiccator until characterisation.

Six specimens with dimensions 2 mm x 2 mm x 25 mm were fabricated from each type of composite fibres, by thermoplasticising the materials in a standardised stainless steel heated mould (Fig. 2.2-2). This mould was specially designed to fit on the thermal probe of the extruder and heat the material to the desired temperature, which was fixed at 190-200 °C (Jaggi *et al.* 2012).

Figure 2.2-2 Schematic diagram illustrates the processing of PE-HA composite fibres

2.2.1.3 Characterisation of the composites

2.2.1.3.1 Flexural properties

The three-point bending test was used to measure the flexural strength and flexural modulus of the composite materials at room temperature, as described in section 2.1.2.6.

2.2.1.3.2 Thermal characterisation

Differential scanning calorimetry (DSC) was carried out using a Perkin Elmer machine (Waltham, MA, USA) (Fig. 2.2-3) to measure crystallinity and melting behaviour of the composites (the technique was described in section 2.1.2.5).

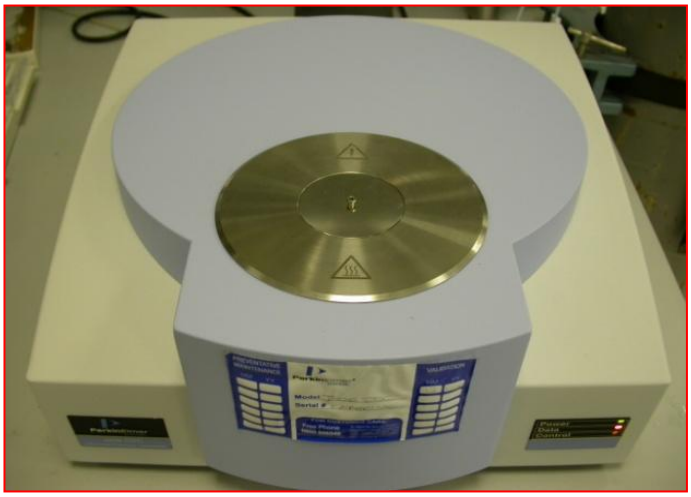


Figure 2.2-3 Perkin Elmer DSC machine

The degree of crystallinity (W*c*) was determined from the following equation:

$$Wc = \frac{\Delta Hf}{\Delta Hf^{\circ}} \times 100$$

Where ΔHf is the enthalpy of melting (the heat of fusion obtained after integrating the area under the normalised melting curve) of the sample and ΔHf° is the melting enthalpy of 100% crystalline LDPE taken as 293 J/g.

2.2.1.4 Results and discussion

The mean, standard deviation and the significant differences between groups of the flexural strength and modulus of all experimental composites are shown in Table 2.2-1. The silanation of the filler had a significant effect on the flexural strength of LDPESHA40 and on the flexural modulus of composite containing 40% and 50% wt. HA, however, it did not result in a significant change in the flexural strength of other composites.

The flexural strength and more apparently, the flexural modulus progressively increased with the increase in HA content up to 40% wt. The statistical analysis showed that the flexural modulus of all silanated composites and the flexural strength of LDPESHA40 and LDPESHA50 composites were significantly higher (P<0.05) than those of pure LDPE.

Table 2.2-1 Formulation and flexural properties of the experimental composites

Designation	Formulation			Flexural properties	
	LDPE powder (wt. %)	HA (wt. %)	Silane coupling	FS in MPa (SD)	FM in GPa (SD)
LDPE	**100**	**0**	**–**	**30.05 (2.1)[a]**	**0.76 (0.12)[a]**
LDPEHA 20	**80**	**20**	**NO**	**34.04 (2.6)**	**2.50 (0.59)[a]**
LDPESHA20	**80**	**20**	**YES**	**34.25 (3.0)**	**2.58 (0.10)[a]**
LDPEHA 40	**60**	**40**	**NO**	**37.77 (1.4)[a,b]**	**2.66 (0.11)[a,b]**
LDPESHA40	**60**	**40**	**YES**	**46.10 (2.2)[a,b]**	**3.09 (0.21)[a,b]**
LDPEHA50	**50**	**50**	**NO**	**34.03 (5.1)**	**2.59 (0.17)[a,b]**
LDPESHA50	**50**	**50**	**YES**	**39.54 (3.6)[a]**	**3.02 (0.17)[a,b]**

Letter a indicates the significant differences for all properties (P<0.05) between filled and pure composites. Letter b indicates the significant differences for all properties (P<0.05) between silanated and non-silanated composites (One-way ANOVA)

The preliminary results obtained from this study indicated that the silanated HA effectively reinforces polyethylene, but increasing the filler concentration made processing more difficult and the optimum properties could be achieved above 40% HA. Further addition of HA resulted in a drop in the flexural strength as composites exhibited brittle behaviour.

The degree of crystallinity of pure LDPE and LDPESHA composites is presented in Fig. 2.2-4 and Table 2.2-2. The thermogram of pure LDPE showed a melting temperature around 140 °C; although this is relatively high in relation to its lower density, the selected PE has a relatively high Mw (400,000 g/mol.). The high melting peak may originate from the ability of crystallisation with a large number of long chains (larger crystallite size) similar to that of high-density polyethylene. The addition of SHA fillers resulted in a slight shift in melting temperature to a lower value with the absence of any tangible changes in the crystallisation temperatures.

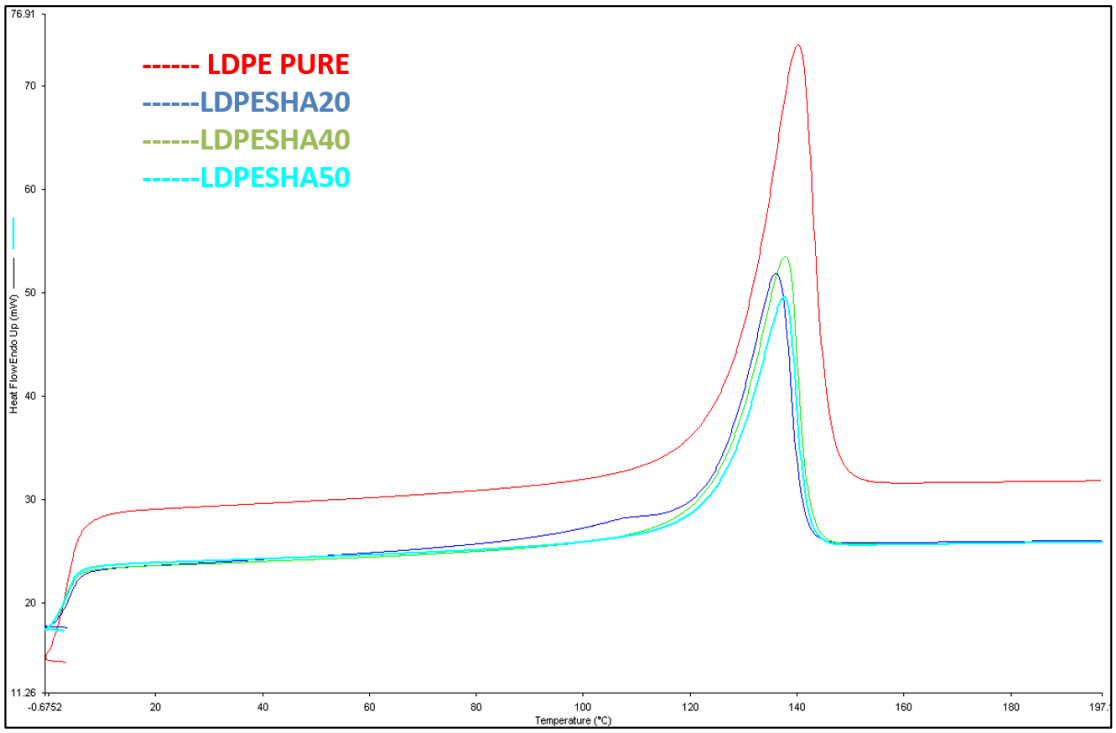


Figure 2.2-4 DSC thermograms of the experimental composites (second heating cycle)

The degree of crystallinity for the composites ranged from 54 to 57% (Table 2.2-2), and the differences were insignificant. This finding indicates the absence of any perceptible/macro-scale changes in the crystalline organisation confirming the thermal stability of the resultant composites. A similar finding has been reported for HDPE/HA bio-composites (Jaggi *et al.* 2012).

Table 2.2-2 Melting behaviour and degree of crystallinity obtained by DSC analysis

Composites	Melting temperature Tm (°C)	Crystallisation temperature Tc (°C)	Degree of crystallinity (W*c*)
Neat LDPE	**140.2 ± 1.0**	**115.0 ± 0.5**	**57 ± 2**
LDPESHA20	**136.0 ± 0.3**	**116.2 ± 0.1**	**56 ± 1**
LDPESHA40	**137.8 ± 0.2**	**115.3 ± 0.3**	**55 ± 1**
LDPESHA50	**137.6 ± 0.3**	**115.5 ± 0.2**	**54 ± 2**

The melting point of the experimental composites at 137 °C meant that the material could be removed easily and safely from the root canal space using a conventional dental heating device.

2.2.2 Retrievability of the experimental PE-HA post

In the incidence of endodontic retreatment and post fracture, post removal should be performed simply with minimal loss of tooth structure and root damage. The time required for a post removal is also of importance in clinical practice. However, the ability to remove an existing post depends on the type of material used.

2.2.2.1. PE-HA post removal protocol

The technical approach for experimental PE-HA post removal is based on the heat and files method (Fig. 2.2-5).

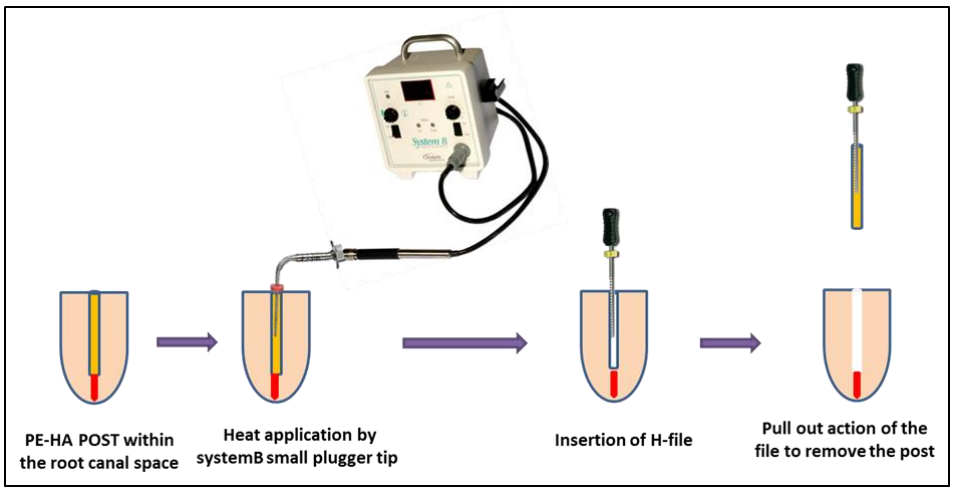


Figure 2.2-5 Schematic illustrating the removal method of experimental PE-HA post

Heat was applied to the coronal and/or middle parts of the post using conventional heat sources such as SystemB heat source (SybronEndo, Orange, CA, USA) with a small size (0.04) plugger tip at a temperature of 135 °C followed by immediate insertion of a matched hedström file. The heat is necessary to thermosoften the post material that creates a gap along its longitudinal axis. Afterwards, the blades of H-files engage in the softened post and effectively auger this material out of a root canal space with a pulling action.

2.2.2.2. A pilot study on PE-HA post removal

Ten single-rooted premolar teeth were sectioned at the cemento-enamel junction and treated endodontically. Then, 9 mm post spaces were prepared, and 10 experimental PE-HA posts (1.5 mm diameter and 20 mm length) were luted with dual-cure resin cement (Clearfil™DC Core plus, Kuraray, Japan) after sandblasting their surfaces with 110 µm aluminium oxide particles for 10 s at 2.8 bar from a distance of 1 cm. The specimens were then embedded in acrylic resin blocks, with use of a parallelometer to maintain parallelism between the posts and resin blocks, and kept humid for 24 h at 37 °C. After storage, the post segments outside the canal were sectioned perpendicularly at the post tooth interface and the remaining post inside the canal was removed using the heat and files method by the same operator. Post removal time (in seconds) was recorded to evaluate efficacy using an electronic watch.

The mean of the time (in seconds) required to remove PE-HA posts (n=10) was 88 ± 23.8. In comparison with the mean removal time of other posts (Table 2.2-3), it seems that the experimental post can be removed easily and effectively, which required less removal time than the currently used fibre posts while preserving tooth structure. The lower melting point of the experimental PE-HA post facilitates its removal with a pre-heated tip at 135 °C, which is considered as a safe temperature since much higher temperatures (200 °C) are applied with these heating devices to apply heat into root canals (Lipski 2005). Although a lower temperature could be used (85 to 100 °C) to soften the PE-HA post material, this may elongate the procedure.

The application of heat disrupts the post structure, especially at the bonding interfaces, leading to post dislodgement. Furthermore, this technical procedure can eliminate the risk of possible perforation or initiation of vertical fractures that may be associated with traumatic root dentine removal.

Table 2.2-3 Mean removal time of different intracanal posts (adapted from literature)

Post type	N	post space length	Luting cement	Removal method	Removal time in Sec (SD)	Reference
Fibre posts (GC Corporation, Japan)	9	9 mm	Resin cement	Largo reamers	594 (204.6)	(Rayyan 2014)
				Roane Gates Glidden drills	883 (646.8)	
				Needle bur #859	665 (264)	
				Post Space Bur #5 length 25	692 (310.8)	
D.T. Light-Fibre Post (RTD, France)	8	10 mm	Resin cement	Ultrasonic handpiece with a #3 post removal tip	201 (13)	(Scotti *et al.* 2013)
Hi-Rem Prosthetic Post				ProFile and #2 Largo drill	37.29 (4.1)	
Titanium posts (ParaPost XP; Coltene)	9	9 mm	Zinc phosphate	Ultrasonic vibration device	101.0 (8.0)	(Ebrahimi Dastgurdi *et al.* 2013)
			Glass ionomer cement		715.4 (67.0)	
			Resin cement		6493 (576.2)	
Nickel-chromium alloy cast post	10	10 mm	Zinc phosphate cement	Ultrasound device with an ST 09 tip	168.5 (23.5)	(Soares *et al.* 2009)
			Glass ionomer cement		59.5 (31.2)	
			Resin cement		285.2 (45.0)	
Ti alloy post (ParaPostXH,Coltene)	10	8 mm	Resin cement	Manufacturer post removal kit (Drills)	840	(Lindemann *et al.* 2005)
ParaPost Fiber White (Coltene)					240	
Luscent Anchors fibre post (Dentatus, NY, USA)					234	
Aestheti-Plus fibre post (Bisco DP, IL, USA)					438	

Chapter 3 Semi-interpenetrating network composites reinforced with Kevlar as intracanal post material for restoration of endodontically treated teeth

3.1 Introduction

The use of an intracanal post in endodontically treated teeth improves the retention of the core, which substitutes the missing coronal tooth structure, and provides support for the final restoration (Schwartz and Robbins 2004). Fibre reinforced composites (FRC) have been used for fabrication of posts to replace the high modulus metallic cast and prefabricated posts that make them less desirable due to the tendency to cause root fractures (Wandscher *et al.* 2014). In the last three decades, glass fibre reinforcements have been documented for providing aesthetic quality in conjunction with high tensile strength and lower modulus (Plotino *et al.* 2007a; Baba *et al.* 2009), however the hydrolytic instability of the silicate glass fibres (Ehrenstein *et al.* 1990) is deemed to cause the mechanical deterioration of these posts. It is imperative that posts are not moisture sensitive, able to exhibit a predictable bonding to tooth tissue, and possess high flexural and fatigue resistance to prevent flexion of the core during parafunctional movement.

In FRC's, the fibres act as an effective reinforcement for the polymer matrix and stress is transferred from the matrix to the fibres. The choice of fibre/matrix and the interfacial bonding are important determinants of the integrity of fibre posts (Behr *et al.* 2000). Several fibres of carbon, glass, quartz, aramid (Kevlar), and ultra-high molecular weight polyethylene (UHMWPE) fibres have been used in dental FRCs with varying degree of success. Among them, the silica and quartz fibres are aesthetically suited that have led to their use in combination with methacrylate or epoxy resin matrices as components of commercial FRC posts. However, the tendency of hydration of silica based glass fibres (Ehrenstein *et al.* 1990) compounded with chemical degradation and plasticisation of the polymer matrix in presence of moisture, compromise the interfacial adhesion between

fibres and the polymer matrix (Abdel-Magid *et al.* 2005). In a clinical situation, although the intracanal posts are entombed by other restorative materials, which prevent or reduce their direct contact with fluids, their structure may still be affected on cyclic loading and the induced stresses. Previous studies have shown that mechanical fatigue during cyclic loading that simulate the normal occlusal and masticatory functions contribute to reduction in mechanical properties of FRC posts after ageing (Drummond and Bapna 2003; Grandini *et al.* 2005a; Cheleux and Sharrock 2009), which renders them flexible and transfer the stress to the post-luting or resin-dentine interface leading to debonding, a frequent failure mode of ETT restored with fibre posts (Rasimick *et al.* 2010).

Kevlar fibres exhibit a unique combination of high strength, modulus, toughness and thermal stability (Hussain *et al.* 2014) and Kevlar composites find extensive use as bullet-proof vests, sporting goods and aviation industry. Kevlar fibres have been used in acrylic dentures to improve fracture and fatigue resistance (Berrong *et al.* 1990; Malicka-Soczka *et al.* 2009), however much less in dental composites due to their inherent yellow colour, which interferes with aesthetic requirements.

The ability of Kevlar fibres to reinforce polymeric matrices prompted the present study and were used to reinforce Bis-GMA/TEGDMA/PMMA semi-IPN matrix with titanium dioxide (TiO_2) nanofiller composite and characterised to function as a new intracanal fibre post material. The surface of Kevlar fibre is highly crystalline, chemically inert and smooth, which necessitates surface treatment to allow an interfacial adhesion between the fibre and the matrix (Wang and Xia 1999; Hussain *et al.* 2014). Thus, the fibres were etched with acetic acid and subsequently subjected to treatment with a silane coupling agent to enhance the matrix-fibre interfacial adhesion. The effect of the action on the surface treatment of the fibres were first assessed by examining the flexural strength of the fibre reinforced composites prepared solely by using the semi-IPN with the untreated, acetic acid etched and etched and silane coupled fibres (the reinforcements) prior to the fabrication of K FRC composites for the intracanal posts. The post materials were

characterised extensively and compared with a commercial glass fibre reinforced semi-IPNs post material (everStick®POST).

3.2 Materials and methods

3.2.1 Materials

Kevlar 49® yarn (polyparaphenylene terephthalamide), 12 µm filament diameter, 1.44 g/cm^3 density was purchased from Goodfellow Cambridge Limited, England. Acetic acid (BDH, VWR, Dorset, UK) and silane coupling agent A-174 (3 trimethoxysilyl propylmethacrylate), (Merck, Frankfurt, Germany) were used for fibre surface treatment. For the semi-IPN system, 2, 2-Bis [4- (2-hydroxy-3 methacryloyloxypropyl) - phenyl] propane (Bis-GMA) and tri-ethylene glycol dimethacrylate (TEGDMA) were purchased from Esschem Europe Ltd. Polymethyl methacrylate particles (PMMA, Mw~350000 g/mol), titanium dioxide (nanopowder, <100 nm particle size) and Benzoyl peroxide (BPO) were supplied by Aldrich, UK. EverStick® posts from Stick, Tech, Turku Finland was used as a commercial reference (Table 3-2).

3.2.2 Surface treatment of Kevlar fibres

Kevlar fibres were first treated with 99.9% acetic acid at 55 °C for 2 h, washed three times with distilled water and dried under vacuum at 50 °C for 8 h (AA treated KF). A part of the AA-treated KF fibres were further subjected to wet silanation using 2% A-174 in a mixture of ethanol-water (90/10 w/w) and then dried under vacuum at 50 °C for 8 h before use (AA-silanated KF). The modified fibres were characterised by ATR-FTIR (ATR-Perkin-Elmer-Spectrum One, Waltham, MA, USA) and spectra recorded in the 4000 cm^{-1} to 650 cm^{-1} region with a wavenumber step of 0.5 cm^{-1}. The morphology of the fibres was visualised by scanning electron microscopy (Hitachi High Technologies, S-3500N, USA) at an accelerating voltage of 10 KeV and a magnification of 1000x. Untreated Kevlar fibre was used as the reference.

3.2.3 Specimen preparation

3.2.3.1 Preparation of Kevlar fibre/semi-IPN matrix reinforcements

Untreated, AA-treated and AA-silanated Kevlar fibres were used to prepare three experimental reinforcements, using semi- IPN matrix system prepared by mixing Bis-GMA, TEGDMA and PMMA at a ratio of 54:36:10 (w/w) respectively. A blend of Bis-GMA/TEGDMA was prepared first in a glass cup. Benzoyl peroxide was added as a heat curing initiator at 1% by weight of the net resin mix. PMMA particles were then added to it and stirred with a magnetic stirrer at room temperature for 24 h to allow swelling or dissolution of the particles. The fibre feed content and matrix was kept constant for the three formulations and bar-shaped test specimens (2 mm x 2 mm x 25 mm) were prepared for three-point bending tests using PTFE moulds. Two Kevlar fibre bundles (25 mm in length), which consisted of 4 sub-bundles of 1000 filaments each, were weighed first and impregnated with a constant amount of resin mix for 24 h. The impregnated bundles were then embedded unidirectionally into the mould with an excess of the resin matrix. The mould was clamped with spring clips between two glass slabs and cured in a heat oven for 1 h at 80 ºC. After curing, the sides of all specimens were polished with a silica paper (grit 800-1200) to improve the surface finish. The test specimens were stored at room temperature for 24 h before three-point bend testing.

3.2.3.2 Preparation of experimental Kevlar FRC and everStick®Post specimens

The post composites were prepared using the AA-silanated K fibres, which yielded the best flexural strength, in combination with the semi-IPN resin mix (prepared in 3.2.3.1) and TiO_2 nanofiller (75/25 wt./wt.), detailed in Table 3-2. Rectangular bar-shaped specimens (2 mm x 2 mm x 25 mm) and cylindrical post samples (2 mm diameter 20 mm long) were fabricated following the above-mentioned method and used for further characterisation (Fig. 3-1).

The fibre content in percentage volume was assessed using the formula (Abdulmajeed *et al.* 2011):

$$V_k(\%) = \frac{D_s - D_r}{D_f - D_r} \text{ X } 100 \qquad (1)$$

where, V_k is the vol.% of the KF, D_s is the density of the K FRC sample, D_r is the density of resin matrix and D_f is the density of the KF (1.44 g/cm^3). This method determines the densities (calculated by Archimedes' principle) of FRC sample and resin composite matrix samples by measuring their mass in air and water. An analytical balance (Mettler, Toledo, AG64, Switzerland) equipped with a density kit was used for the measurements.

The everStick® material was used as received and handled according to manufacturer's instructions. To prepare a bar-shaped specimen, two unpolymerised bundles of the material (each containing about 4000 glass fibres) were rolled together and embedded into a PTFE mould covered with Mylar strips, clamped between two glass slides and then cured by visible light for 40 s each side, using Optilux 501 (Demetron, Danbury, USA) dental curing unit with an irradiance of 650 ± 50 mW cm^{-2}, verified before the curing using a radiometer. After manual light-curing, the specimens were further polymerised in a light-curing oven with heat (Licu Lite; Dentsply DeTrey, Dreieich, Germany) for an additional 20 min (Lassila *et al.* 2005).

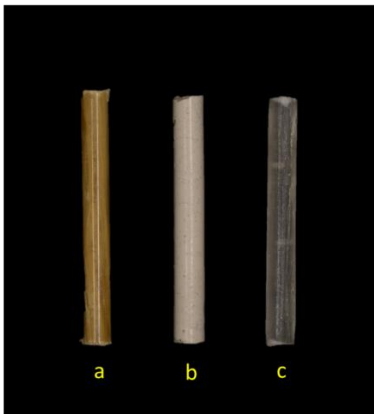


Figure 3-1 Experimental K FRC posts (a) without TiO_2 nanofiller and (b) with TiO_2 nanofiller, the inclusion of TiO2 offsets the yellow colour of Kevlar and (c) everStick® commercial post

3.2.4 Flexural properties of KF reinforcements and FRC post materials.

Three-point bending tests were carried out according to ISO 10477 standards (ISO10477) at a cross-head speed of 1 mm/min and span length fixed at 20 mm on a universal testing machine (Instron, M5569A) to determine the flexural properties of K fibre reinforcements, experimental K FRC post and everStick® post materials. To evaluate the effect of thermal stress, test specimens from experimental K FRC post and everStick® post materials were thermo-cycled for 10,000 cycles in water from 5 °C to 55 °C, with a dwell time of 30 s in each bath and a transfer time of 5 s.

Flexural strength (σ) in MPa and flexural modulus (*E*) in GPa were calculated using Eqs. (2) and (3) respectively.

$$\sigma = \frac{3FL}{2bh^2} \quad (2)$$

$$E = \frac{L^3}{4bh^3} \times \frac{F}{Y} \quad (3)$$

where *F* = maximum strength, *L* = distance between the rests, *b* = width of the specimen, *h* = height of the specimen, and *F/Y* = slope of the linear part of the stress– strain curve.

3.2.5 Characterisation of FRC post materials

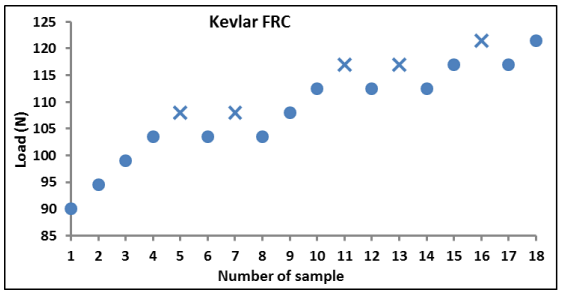
3.2.5.1 Dynamic mechanical analysis (DMA)

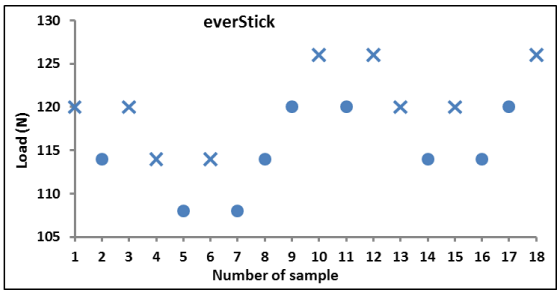
Tan delta measurements were carried out using a DMA7 analyser (Perkin-Elmer, USA) in a three-bending mode at a frequency of 1 Hz and a heating rate of 5 °C/min within 25 °C and 200 °C temperature range.

3.2.5.2 Fatigue test

A fatigue testing machine (Bose, ElectroForce® System, MN, USA) was used to evaluate the flexural strength fatigue limits of the post materials in the three-point bending mode at a frequency of 2 Hz for 5 x 10^5 cycles or until failure. The test was started by loading the first specimen in each group at approximately 60% of its static flexural load obtained

earlier. The staircase method was employed with a stress increment of 4.5 N for Kevlar FRC and 6 N for eveStick (5% of the initial load). Since this specimen did not fail in 5 x 10^5 cycles, the second specimen was stressed with a load one increment higher. If it failed in less than 5 x 10^5 cycles, the next specimen was applied to the test at a stress level one increment lower. This procedure was continued until eighteen specimens of each group were tested. Tests were carried out at a room temperature and kept moist during cycling via continuous irrigation. The data of the staircase method employed for both groups and their analysis are shown in Fig 3-2.





Load (N)	i	ni (failures)	in_i	i^2n_i
121.5	2	2	4	8
117	1	2	2	2
108	0	2	0	0
		N=Σ ni = 6	A=Σ in_i =6	B=Σ i^2n_i =10

Load (N)	i	ni (failures)	in_i	i^2n_i
126	2	3	6	12
120	1	4	4	4
114	0	2	0	0
		N=Σ ni = 9	A=Σ in_i =10	B=Σ i^2n_i =16

Figure 3-2 Staircase method with data analysis required to determine the mean fatigue limits and standard deviation at 5 x 10^5 cycles for K FRC and everStick®. X: Failed specimen, ●: Non-failed specimen

The mean fatigue limit and standard deviation for each group were calculated using the formulae reported by Draughn (Draughn 1979).

$$\bar{X} = X_0 + d\,[A/N \pm ½] \quad (4)$$

$$SD = \langle 1.62\, d\, [(NB - A2)/N2] + 0.029 \rangle \quad (5)$$

Where, $\bar{X}$ is mean fatigue strength limit; X_0 is the load at the lowest stress level at which the fracture or non-fracture of specimens occurred; d is the stress increment; SD is the standard deviation. In formula 1, the positive sign is used when the analysis is based on non-failed specimens, and the negative sign used when failed specimens were considered.

3.2.5.3 Water sorption and solubility

Cylindrical specimens (2 mm diameter x 20 mm length) were initially weighed (Mi) to an accuracy of ± 0.0001 g (Mettler, Toledo), and then kept in individual containers in 10 ml deionized distilled water at 37 °C. Water uptake was recorded at regular interval during 28 days until there was no significant change in the mass (M_s). A desorption cycle was carried out at 37°C to a constant dry mass (M_d). The water sorption (W_{SP}) and solubility (W_{SL}) in µg/mm³ were calculated using the following equations:

$$W_{SP} = M_s - M_d / V \quad (6)$$

$$W_{SL} = M_i - M_d / V \quad (7)$$

where, V is the volume of the sample.

3.2.5.4 Radiopacity

Five posts (2 mm diameter × 20 mm length) from both groups were digitally photographed alongside a high purity aluminium step-wedge (1100 alloy) with thickness varying from 1 to 10 mm with increments of 1 mm as a reference according to ISO 4049. The images were taken using a dental X-ray unit (Heliodent; Sirona, Bensheim, Germany) operating at 70 kV, 8 mA, and 0.2 seconds) with a phosphor plate system (Digora® Optime; Soredex, Tuusula, Finland). ImageJ processing and analysis in java, version 1.47v was used to measure the grey value of the sample and aluminium in the resulting images.

3.2.5.5 Cytocompatibility

The cytocompatibility of the post materials was evaluated using human gingival fibroblast (HGF) cells (obtained at passage number P8) taken from ScienCell™ research laboratories, UK. The cells were cultured at 37 °C humidified atmosphere with 5% CO_2 in fibroblast medium (FM-2301, ScienCell™, UK) consisting of 500 ml basal medium, 10 ml of fetal bovine serum (FBS, Cat.no. 0010, 5ml of fibroblast growth supplement (FGS, Cat.no. 2352) and 5 ml of penicillin/streptomycin solution (P/C Cat.no. 0503).

MTT assay was used to evaluate cell viability at 24 h and 48 h according to the International Standard (ISO10993-5). The specimens were first sterilised with 70% ethanol in water and then with ultraviolet irradiation. Composites eluents were obtained by immersing the samples in 3 ml of sterile fibroblast medium within bijou vials, which were then placed onto a roller at room temperature. The supernatants were collected at 24 and 72 h time points and refrigerated at -20 °C to be used for cytotoxicity measurements. HGF cells were cultured at 37 °C humidified atmosphere with 5% CO_2 to reach about 80% confluent, trypsinised and then seeded on a 96 well plate (100 µL/well) at a density of 1 x 10^4 cells/well. The cells were incubated at 37 °C, 5% CO_2 for 24 h to allow for cell to attach and acclimatisation prior to addition of the test eluents. After 24 h, the fibroblast media were removed from both plates and replaced with 100µl of the leached eluents from composites. Untreated cells served as a negative control while positive control cells were treated with 10% v/v ethanol solution. Each group consisted of five replicate wells. Then the plates were incubated for 24 h or 48 h (exposure times), after which the test eluents were removed and replaced with 100 µL of MTT (5 mg/mL PBS) for 4 h. MTT solution was then removed and 100 µL dimethyl sulfoxide (DMSO) was added to each well. The plate was shaken for 5 min and the absorbance of the purple coloured solution was measured using a UV–visible spectrophotometer plate reader at wavelength 570 nm (Opsys MR, Dynex Technologies, Chantilly, VA, USA). Relative cell viability is expressed as a percentage of untreated negative control reading. Each experiment was done in duplicates.

3.2.6 Statistical analysis

After analysing the normality of data distribution, a one-way ANOVA and Tukey's post-hoc test were employed to determine the effects of different surface modifications on flexural properties of KF reinforcements. A Mann-Whitney (non-parametric) test or Independent *t*-test (for normally distributed values) was used to detect the other variances between the experimental and commercial composites tested. In all tests, the level of significance was set at $P<0.05$.

3.3 Results

3.3.1 Kevlar fibre surface modification

The FTIR spectra of the untreated Kevlar fibre showed three main peaks at 3313 cm^{-1}, 1640 cm^{-1} and 1538 cm^{-1}, arising due to the –NH– stretching, conjugated –C=O stretching and –NH– bending respectively. The absorption bands at 1511 cm^{-1}, 1017 cm^{-1} and 820 cm^{-1} are due to the (C-H) of the aromatic ring. The acetic acid treatment of the fibres led to the appearance of low intensity peak at 1718 cm^{-1} corresponding to the C=O stretching band and the broad bands between 2800 and 3000 cm^{-1} of the OH- and CH-stretching, which clearly indicated that the surface was modified. The spectra of the AA-silanated KF showed the appearance of a new absorption band at 1718 cm^{-1}, corresponding to the carbonyl stretch, whilst the peaks at 3313 $cm^{-1}$and at 1538 cm^{-1} due to the –NH groups disappeared by virtue of the silanation (Fig. 3-3). This confirmed that the silane coupling agent reacted through the N-H group of the KF.

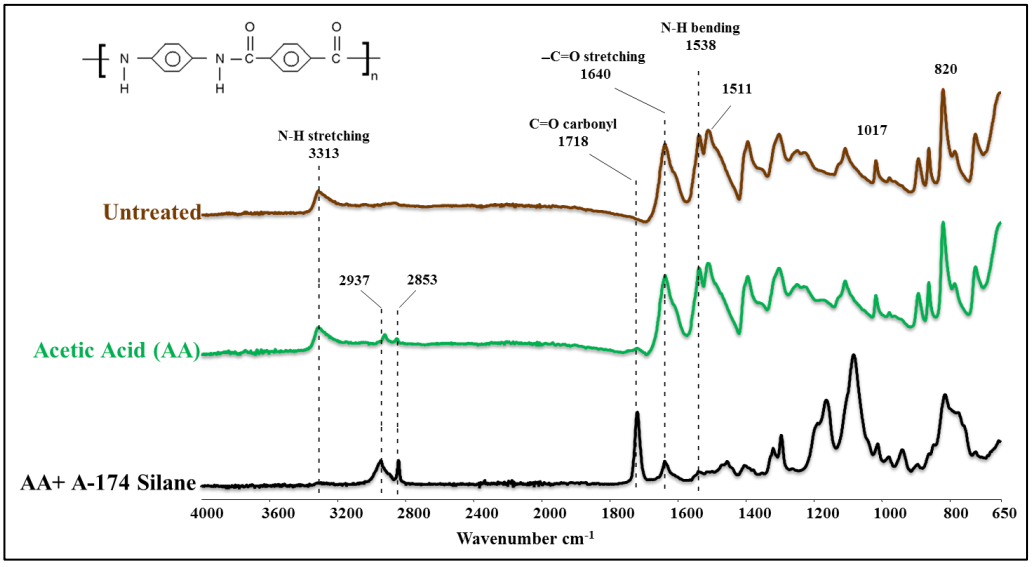


Figure 3-3 FTIR spectra of virgin and modified Kevlar fibres

The surface morphology examined by scanning electron microscopy showed that the untreated fibres (Fig. 3-4a) had a smooth texture whilst the AA-treated fibres appeared to be etched with a rough surface (Fig. 3-4b). However, on silanation of the AA-treated fibres, a smooth surface reformed with some blisters (Fig. 3-4c) probably associated with the underlying etched surface.

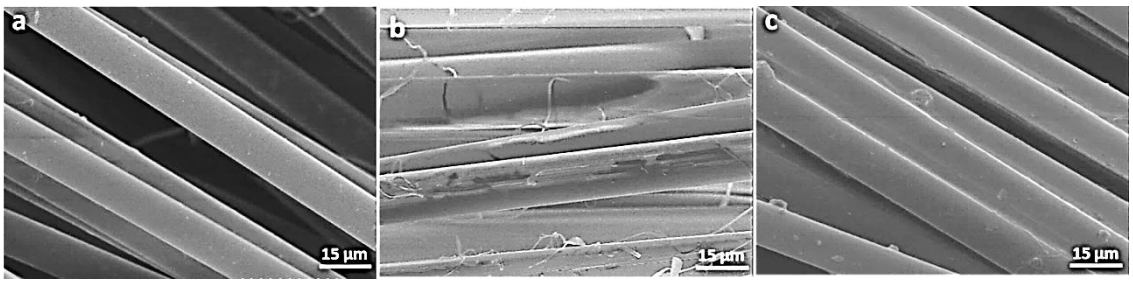


Figure 3-4 SEM images of Kevlar fibres at 1000x.Untreated KF: showed typical Kevlar features, which has a relatively smooth surface with some small impurities; (b) AA-treated KF: the surface of the fibre became rougher and more blisters and pimples produced in etching; (c) AA-silanated KF: a coat of silane agent covered the surface of the fibre and some blisters are clearly visible

The influence of fibre surface modifications on flexural properties of KF/matrix reinforcements is shown in Table 3-1. A statistically significant increase in the flexural strength was observed in the AA-silanated KF containing composites in comparison to the untreated fibres, however, there were no differences in their flexural moduli.

Table 3-1 Flexural strength (FS) and flexural modulus (FM) of the semi IPN of BisGMA/TEGDMA/PMMA reinforced with Kevlar fibres subjected to different surface modifications [mean (SD), n=6]

KF surface modification	Flexural properties of KF reinforcement	
	FS in MPa (SD)	FM in GPa (SD)
Untreated KF	**283.2 (47.7)***	**15.4 (1.3)**
Acetic acid treated KF	**340.0 (36.6)**	**15.3 (1.7)**
Acetic acid + A-174 silanated KF	**394.0 (43.4)***	**16.5 (2.9)**

***Differences were statistically significant (*P*< 0.05)**

3.3.2 Characterisation of FRC post materials

3.3.2.1 Flexural properties

The flexural modulus (FM) of the experimental K FRC was comparable to that of the commercial material, whilst its flexural strength (FS) was significantly lower (P<0.05), detailed in Table 3-2. Thermo-cycling in water significantly decreased the flexural strength of both FRC materials whilst it had no significant effect on their flexural modulus (P>0.05). However, the reduction in FS of K FRC (16%) after thermal cycling was much lower than that of everStick® (35%).

Table 3-2 Flexural strength (FS) and flexural modulus (FM) of experimental Kevlar FRC and commercial everStick® post materials under static and thermal-cycled conditions [mean (SD), n=6]

FRC group	Description	FS in MPa (SD)		FM in GPa (SD)	
		Static	Thermal cycled	Static	Thermal cycled
Experimental K FRC	**Two bundles (4000 filaments each, 12 µm diameter) of silanised pre-impregnated Kevlar fibres in Bis-GMA/ TEGDMA/ PMMA semi-IPN matrix with 25% wt. TiO_2 nanofillers. Fibre content: 32 vol.%. Heat-polymerisation**	**450.0 (35.9)**	**378.4* (33.6)**	**18.9 (1.6)**	**18.0 (1.5)**
Commercial everStick® Patch No.: 2050426-ES-125	**Two bundles (4000 filaments each, 15 µm diameter) of silanised pre-impregnated unidirectional glass fibres in Bis-GMA/PMMA semi-IPN matrix. Fibre content: 48 vol.%. (Information provided by manufacturers) Light polymerisation + Oven (light/heat) polymerisation**	**583.0 (39.5)**	**381.7* (53.5)**	**19.2 (2.5)**	**17.7 (2.1)**

***Differences were statistically significant (*P*<0.05) between static and thermal-cycled conditions within the same group**

3.3.2.2 Dynamic mechanical analysis (DMA)

Fig. 3-5 illustrates the evolution of tan *δ* with temperature for the experimental K FRC and the commercial everStick® materials. The peaks in the tan *δ* curves correspond to the drop in the storage modulus (*E'*) curves and represent the *T*g values for the composites. As the temperature was increased, the K FRC showed a steady reduction in *E'* value and a relatively constant tan *δ* value, with a broad peak at ~155.2 °C, whilst everStick® exhibited a sharp decrease in *E'* value at higher temperature and the tan *δ* curve appeared to peak at 87 °C.

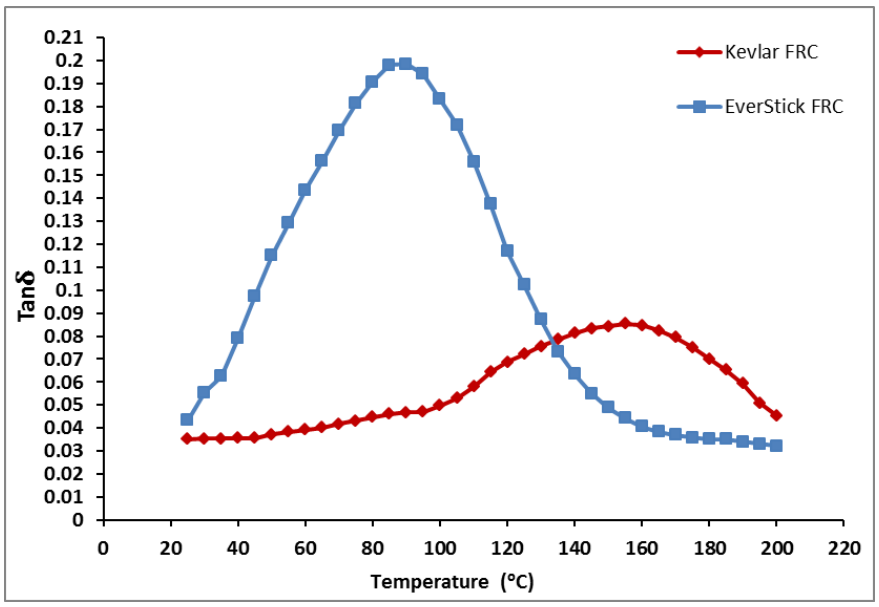


Figure 3-5 DMA curves showing the temperature dependence of tan δ of the experimental composite and commercial material. The tan δ for everStick® increased sharply with temperature comparing to Kevlar FRC; both materials show one relaxation peak (*T*g). The low value tan δ of Kevlar FRC at the *T*g indicates better interfacial adhesion between the fibres and the matrix

3.3.2.3 Fatigue test

The static flexural strengths versus its fatigue limits at 5 x 10^5 cycles of FRC's results are shown in Fig. 3-6. The flexural fatigue limit of the experimental K FRC was about 73% of the static FS, whilst that of everStick® material was about 58% of the static value.

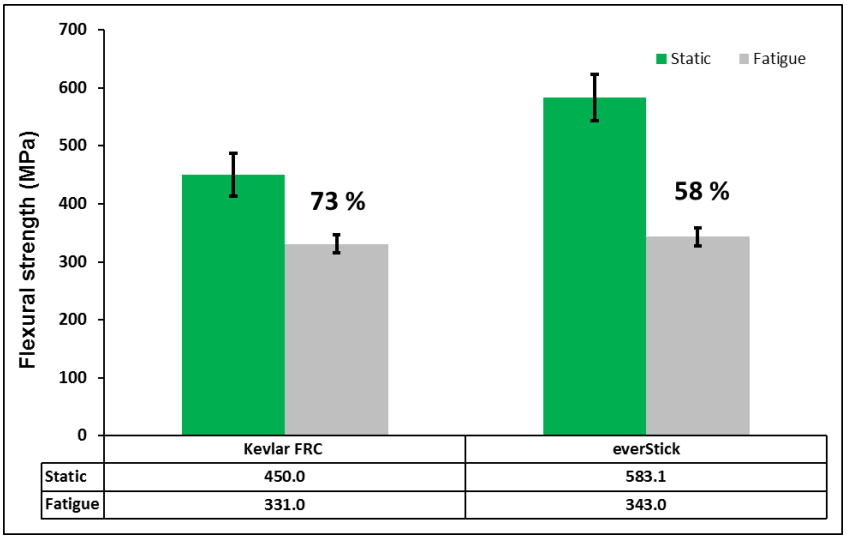


Figure 3-6 Flexural strength fatigue limit at 5 x 10^5 cycles for K FRC and everStick® material

3.3.2.4 Water sorption and solubility

No statistically significant difference (*P*>0.05) in water uptake measured up to 28 days was observed between K RFC and everStick®, however, the solubility of everStick® was significantly higher than that of the experimental K FRC (Table 3-3).

Table 3-3 Water sorption (Wsp) and solubility (Wsl) after 28 days immersion period in distilled water [mean (SD), n=5]

FRC group	Wsp in µg/mm^3 (SD)	Wsl in µg/mm^3 (SD)
Kevlar FRC	**53.3 (3.0)**	**5.4 (0.67)**
everStick®	**47.24 (5.0)**	**27.2 (8.3)***
***Differences were statistically significant (*P*<0.05).**		

3.3.2.5 Radiopacity

The radiopacity of the experimental and commercial posts with the grey-scale value of aluminium step wedge measurements in (mm AL) shown in Fig 3-7 indicate that K FRC posts exhibited significantly higher radiopacity (3.9 ± 0.3 mm Al) than everStick® post (*P* <0.05) at about 0.9 ± 0.1 mm Al.

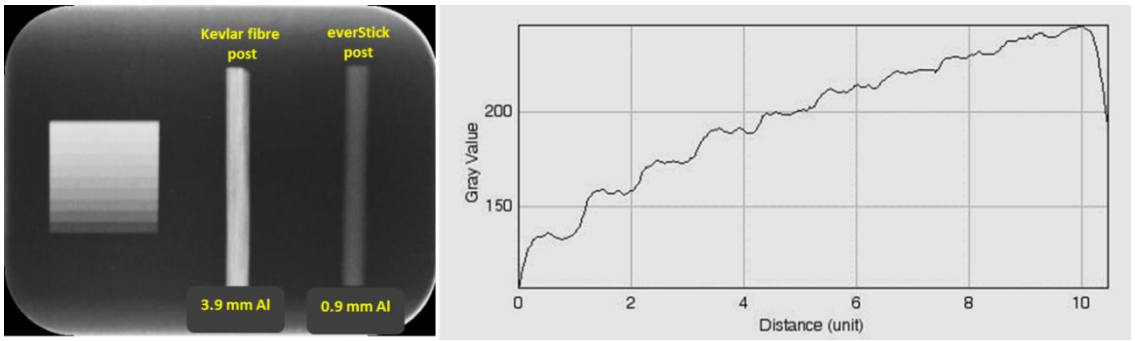
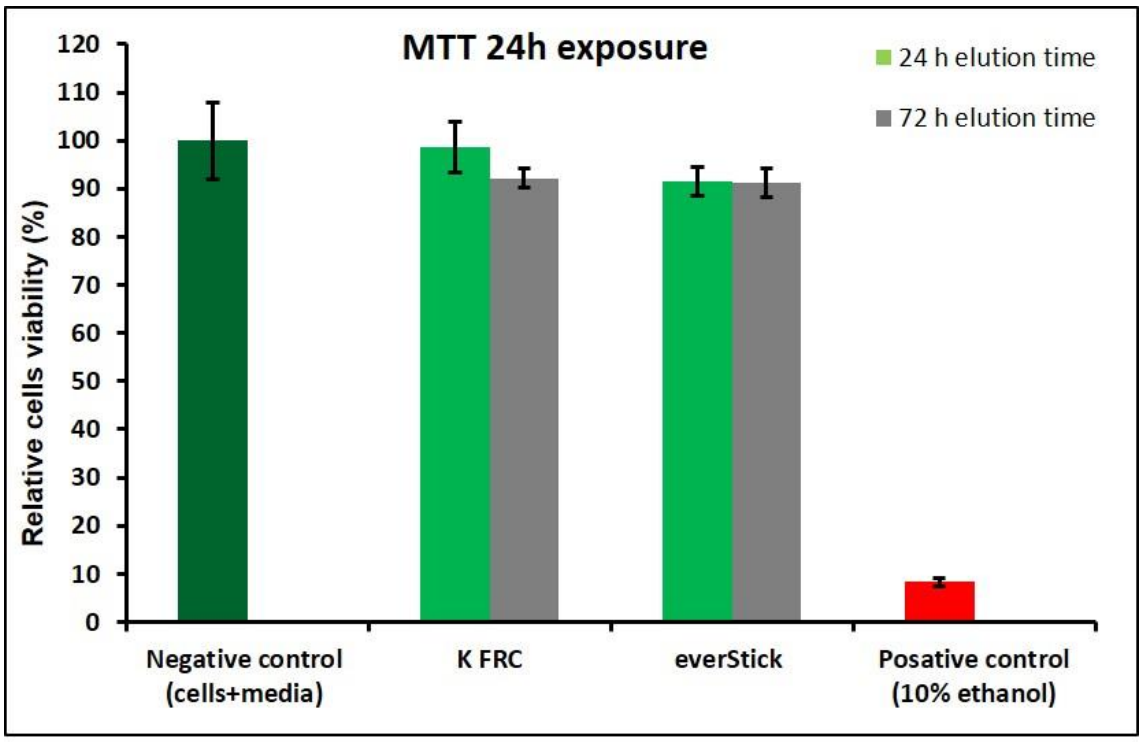


Figure 3-7 Representative radiographs of experimental K FRC and everStick® posts in relation to the density of the aluminium step wedge

3.3.2.6 Cytotoxicity

The MTT assay results demonstrated that extracts from K FRC exposed for 24 and 48 h exhibited no significant reduction (about 10%) in cell viability in comparison to the negative control. No statistically significant different were observed with everStick®, however as expected the positive control had a 90% reduction in cell viability (Fig. 3-8).



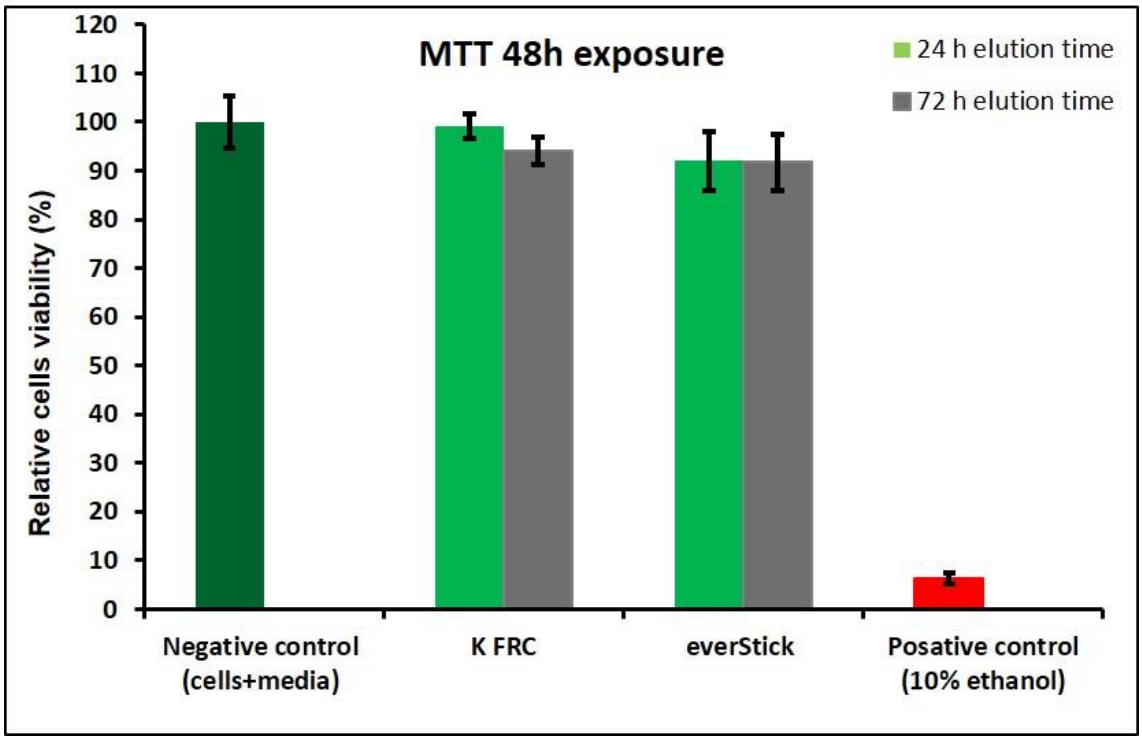


Figure 3-8 The relative cell viability measured by MTT absorbance and presented as percentage of negative control group which was set to represent 100%. Values are the average of five replicate wells and expressed as mean and SD

3.4 Discussion

The fibres play an important role in improving the mechanical properties of FRC's and enable the transfer of stresses from the weaker matrix under applied loads (Khan *et al.* 2015). FRC posts with clear glass fibre reinforcements have gained popularity among clinicians, however, longitudinal studies report various degrees of failure rates ranging between 12.8% at 2 years and 32.5% at 6.5 years (Naumann *et al.* 2005; Naumann *et al.* 2008). The harsh oral environment adversely affects the mechanical properties of fibre posts, which in turn shorten the clinical durability of the restorations supported by fibre posts leading to decementation.

Considering the biomechanical behaviour and need of hydrolytic stability we reported the use polyethylene-hydroxyapatite (PE-HA) thermoplastic based composite fibres as potential new post material (Almaroof *et al.* 2015), however, the structural flexibility of the composite fibres limit their use in small diameters due to the significantly lower strength compared with metal and some fibre posts. Kevlar, on the hand, has extremely good mechanical properties, however, its use in endodontic fibre posts is very limited with one study by Kim *et al.* (Kim *et al.* 2011), where Kevlar, glass and UHMWP fibres were used to reinforce thermosetting resin matrices fabricating three kinds of experimental FRC posts. The flexural properties of Kevlar posts obtained were between glass and polyethylene, however, the high performance of the Kevlar fibres with respect to high tensile strength and modulus was not fully utilised because of the surface inertness (Xu *et al.* 2016).

Thus, the Kevlar fibres in this study were pre-treated with acetic acid alone and acetic acid followed by silanation, especially as it creates an acid etching effect, which improves the interfacial adhesion with the matrix (Yue and Padmanabhan 1999). The etching of the K fibres was apparent from the SEM and FTIR spectra, however, detection of a few carbonyl groups present was difficult due to masking by the high intensity peaks arising from Kevlar itself (Fig. 3-3). However, the subsequent coupling on pre-treated Kevlar

fibres clearly showed the peaks due to carbonyl absorption at 1715 cm^{-1} confirming the successful chemical attachment of the silane coupling agent on the surface of the fibre (Andreopoulos 1989; Ou *et al.* 2010).

The flexural strength of the composites increased with the AA-treated fibre composites, which was attributed to mechanical interlocking resulting from surface micro-roughness of the fibres post etching. The subsequent silane coupling agent resulted in considerable chemical changes on the surface of the fibres, efficiently bound the modified fibres to the polymer matrix upon polymerisation. Therefore, Kevlar/semi-IPN matrix reinforcements with superior interfacial and flexural properties were developed by using the combined benefits of chemical and physical changes obtained with these surface treatment procedures that are compatible with the resin matrix.

The rationale behind the use of semi-IPN structures, which are formed by the polymerisation of the cross-linking monomers (Bis-GMA/ TEGDMA) in the presence of solvated linear polymers PMMA, is to improve both mechanical (Lassila *et al.* 2005) and chemical adhesion between polymer-based materials (Bell *et al.* 2005). The absence of a chemical union between the methacrylate-based resin composite and epoxy resin matrix of the fibre posts is the primary cause of weakness in post-to-composite bonds (Monticelli *et al.* 2008). Epoxy polymers exhibit a high degree of conversion and highly cross-linked structures. The use of semi-IPN technology has been claimed to be an effective method to enhance the adhesion of glass fibres to Bis-GMA matrix (Lastumäki *et al.* 2003). Consequently, an individually formed and *in situ* polymerised glass FRC post (everStick®) has been introduced by Stick Tech, which is used as a control in the present study. EverStick® and other translucent posts have the light- transmitting ability that increases the depth of cure of the light cured resins (Lui 1993) and consequently improves their mechanical properties. However, the efficiency of this propagation, especially in the deeper regions of the canal is yet to be investigated (Salomao *et al.* 2015). No significant difference in root dentine bond strength values between translucent and opaque fibre posts were shown in albeit one study (Mallmann *et al.* 2007). On the

other hand, the use of newly developed dual-cured or self-curing composites in fibre posts cementation is highly recommended to secure polymerisation in deep regions (Mainardi *et al.* 2015).

TheTiO_2 nanofillers incorporated in the final post formulation distinctly contributed to off-setting the yellow colouration (Figure 3-1) due to Kevlar fibres and additionally enhanced the radiopacity, an important requirement for endodontic posts. The weight fraction of the filler was selected on the basis of optimal mechanical properties and radiographic appearance. The experimental K FRC exhibited excellent radiopacity (3.9 mm Al equivalent) since the radiodensity of a post material needs to be higher than that of dentine (1 mm Al) and similar to or slightly greater than that of enamel (2 mm Al) to facilitate their radiographic detection (Hitij and Fidler 2013). In contrast, everStick® posts failed to meet this requirement with significantly lower value at 0.9 mm Al, which was similar to that reported by Dündar *et al*. (Dündar *et al.* 2011b). The titanium oxide also increased the flexural strength and modulus by about 14% (Table 3-2) due to the dispersion of these particles between fibre filaments, which increases the bending resistance.

A comparison of the experimental posts under static conditions indicated the flexural modulus were similar to that of human dentine (17.5 ± 3.8 GPa) (Plotino *et al.* 2007a). However, the flexural strength of K FRC with 32 vol.% fibre content (experimental measurement) was significantly lower when directly compared with everStick® with 48 vol.% (manufactured information) fibre content. The differences in material compositions and their fabrication processes with a lower concentration of fibre content may explain this observation (Abdulmajeed *et al.* 2011). The controlled manufacturing process also enables incorporation of higher fibre content with fewer voids and defects in contrast to manual adaptation (Behr *et al.* 2000).

Thermo-cycling is used as a clinically relevant testing method, representative of the hot-wet conditions in the oral cavity. The thermo-cycled posts exhibited a significant

reduction in FS, consistent with literature findings (Drummond and Bapna 2003; Barbizam and White 2014), however K FRC exhibited a decrease of only 16% compared to 35% of everStick® (Table 3-2), indicating greater thermal stability (good hot–wet properties), which is expected to enhance clinical longevity. Since KF fibres have superior thermal stability in comparison to glass (Wang and Xia 1999) and KF composites have better resistance to water (Ellakwa *et al.* 2002), the lower extent of damage of the K FRC post thermo-cycling is indicative of the significant contribution of the Kevlar fibres. Hydrolytic degradation induced by leaching of glass forming oxides can weaken the silane-promoted adhesion between the glass fibre and polymer matrix (Lassila *et al.* 2002), hence are less resistant moisture, especially at higher temperatures. These results are also in agreement with the water sorption data that also showed a high level of solubility in everStick® indicating the elution of leachable components (unreacted monomers) even on 28 days storage in water.

Under cyclic loading, restorative materials can fail considerably below their ultimate flexural strength measured in static conditions. A staircase method was used to determine the fatigue limit of each composite and the stress was defined as the stress below which failure would not occur in 5×10^5 load cycles. Despite the manual fabrication technique, the experimental K FRC exhibited a fatigue limit of about 73% of its static FS value. This limit was within the range of other dental FRC materials reported earlier (Bae *et al.* 2004) but was significantly higher than that of everStick® (58%). The repeated loading, which weakens the fibre-matrix interfacial bonding and/or propagates a potential area of weakness (voids or cracks), affects fatigue life. The choice of fibre type and wetting resin is known to significantly influence fatigue life. Since both the experimental and commercial composites were based on IPN's the essential differences were the type of fibre reinforcements, additional use of a nanofiller and manual versus mechanical fabrication. The everStick® system has been reported to form an *in situ* semi-IPN nano-interface between the matrix and the fibre improving the mechanical properties in FRC materials. This allows the assumption that the superior performance of K FRC might be

a result of the improved interfacial adhesion between fibre and matrix due to the efficient surface treatment process and in addition the presence of the titanium oxide nano particles, which may have decreased voids and defects. The high fatigue resistance is considered as a significant factor in determining the long-term success of endodontically treated teeth as it is one of the main causes of failure of dental restorations (Dietschi *et al.* 2007; Tang *et al.* 2010).

The dynamic mechanical analysis was performed to evaluate the viscoelastic properties of post composites. As shown in Fig. 3-5, the variations of tan δ with temperature, which reflects the changes in modulus of the resin phase, are markedly different between the materials. For the experimental K FRC, a broader tan δ peak (Tg) at ~155.2 °C was observed and the value of tan δ at this peak was significantly lower than that of everStick® material which appeared to peak at 87 °C. The lower tan δ values of K FRC at temperature range experienced in the oral cavity and the higher Tg value indicate the ability of post material to retain its dimension when stresses are applied. The presence of nanofiller particles in the composites may introduce a broadening of the tan δ curve (Karabela and Sideridou 2011), whilst the extended transition region results from the high degree of structural heterogeneity and crosslinking of the polymeric network (Kannurpatti *et al.* 1998; Lu *et al.* 2001). Here, the molecular chain interlock effect between linear PMMA and cross-linked resin of the experimental semi-IPN presented considerable advantage over the network of everStick® commercial material. The synergistic effect induced by the forced compatibility of individual components in IPN-based composites plays a critical role in determining their thermal stability and mechanical properties (Su *et al.* 2014).

It is important to note that, the *in situ* light-polymerisation of everStick® post could also result in inferior mechanical properties (Lassila *et al.* 2005), which adversely affect clinical durability (Cagidiaco *et al.* 2008a). However, in this study everStick® test specimens were additionally polymerised in a light-curing oven with heat to allow further

conversion of unreacted methacrylate within the polymer matrix (Lassila *et al.* 2005). A lower *T*g value ~55 °C of everStick® material has been reported when polymerised by light only (Khan *et al.* 2008), with the risk of loss in dimensional stability inside the oral environment if exposed to a higher temperature exceeding its *T*g.

The biocompatibility of KF and KF reinforcements has been previously reported in literature (Chen *et al.* 2001), the cytotoxicity of the K FRC as a new combination from treated KF and semi-IPN matrix was evaluated by *in vitro* MTT assay. K FRC showed no release of leachable toxic components. The cell viability was higher than 90% and comparable to that of everStick® both at 24 and 48 h suggesting the non-toxic nature of the material. Here, the use of a methacrylate based resin rather than epoxy resin as a matrix is advantageous as it overcomes any potential toxicity arising from bisphenol A, an endocrine disrupting agent (Rochester 2013), the most commonly used polymer matrix of commercially available FRC posts.

This study provides a new option for intracanal posts with the experimental K FRC materials exhibiting advantages over everStick® material in terms of mechanical stability, fatigue resistance and radiopacity. Further studies employing stress analysis and bond strength tests are necessary for the clinical application of these materials.

3.5 Conclusions

The successful surface treatments of Kevlar fibre with AA and silane coupling agent enhanced its reinforcing effect and verify its feasibility in fibre post fabrication. The compatible combination of treated KF and semi-IPN based composite results in development of new FRC with enhanced interfacial adhesion exhibit appropriate properties as intracanal post material with respect to mechanical stability and fatigue resistance as well as favourable radiopacity and cytocompatibility.

Chapter 4 Imparting the antimicrobial function of eugenol into resin composite materials for intracanal post placement

The second aim of the current research was to impart an antibacterial property into the restoration of ETT to reduce the risk of reinfection and secondary caries, thereby enhancing teeth longevity. In this chapter, the development and characterisation of intrinsically antibacterial resin composites based on a polymeric derivative of eugenol (EgMA monomer) are described in three complemented sections. The first section (4.1, Almaroof *et al.* 2016a) involves the synthesis and characterisation of EgMA monomer, formulation of the experimental composites and the evaluation of their curing kinetics physical and mechanical properties. In the second section (4.2, Almaroof *et al.* 2016b), the antibacterial activity of these composites is reported in conjunction with their behaviour and wettability. Further supplementary information on the bonding performance to the root canal dentine and possible cytotoxicity of these composites are presented in section 4.3.

4.1 A resin composite material containing an eugenol derivative for intracanal post cementation and core build-up restoration

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Authors' contribution

I contributed to the conception and experimental planning of the work, and data analysis. I performed all experiments, designed the manuscript and wrote the paper. Dr Deb contributed to conception and data analysis and critically revised the manuscript. Dr Rojo took part in preparation and NMR characterisation of the monomer and edited the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Abstract

Objectives. To formulate and evaluate new dual cured resin composite based on the inclusion of eugenyl methacrylate monomer (EgMA) with Bis-GMA/TEGDMA resin systems for intracanal post cementation and core build-up restoration of endodontically treated teeth.

Methods. EgMA was synthesised and incorporated at 5% (BTEg5) or 10% (BTEg10) into dual-cure formulations. Curing properties, viscosity, *T*g, radiopacity, static and dynamic mechanical properties of the composites were determined and compared with Clearfil™DC Core-Plus, a commercial dual-cure, two-component composite. Statistical analysis of the data was performed with ANOVA and the Tukey's post-hoc test.

Results. The experimental composites were successfully prepared, which exhibited excellent curing depths of 4.9, 4.7 and 4.2 mm for BTEg0, BTEg5 and BTEg10 respectively, which were significantly higher than Clearfil™DC. However, the inclusion of EgMA initially led to a lower degree of cure, which increased when measured at 24 h with values comparable to formulations without EgMA, indicating post-curing. The inclusion of EgMA also lowered the polymerisation exotherm thereby reducing the potential of thermal damage to host tissue. Both thermal and viscoelastic analyses confirmed the ability of the monomer to reduce the stiffness of the composites by forming a branched network. The compressive strength of BTEg5 was significantly higher than the control whilst flexural strength increased significantly from 95.9 to 114.8 MPa (BTEg5) and 121.9 MPa (BTEg10). Radiopacity of the composites was equivalent to ~3 mm Al allowing efficient diagnosis.

Significance. The incorporation of EgMA within polymerisable formulations provides a novel approach to prepare reinforced resin composite material for intracanal post cementation and core build-up and the potential to impart antibacterial properties of eugenol to endodontic restorations.

4.1.1 Introduction

The restoration of endodontically treated teeth (ETT) remains a challenge in clinical practice, especially under conditions of extensive root canal flaring (Dietschi *et al.* 2008; Coelho *et al.* 2009). Factors such as caries, trauma to immature permanent teeth, anomalies, internal resorption, and over preparation may result in flared root canals with thin dentinal walls and open apices which make root canal debridement difficult and complicate the endodontic and restorative procedures (Mente *et al.* 2009; Cehreli *et al.* 2011). In such cases, prefabricated fibre posts are often used to provide retention for the final coronal restoration. For luting procedures, the use of resin composite core materials with high modulus of elasticity is highly recommended because it can increase the fracture resistance of these weakened teeth and is an alternative to resin cements for one-stage post placement and core build-up restoration (Boschian Pest *et al.* 2002; Naumann *et al.* 2011). The modulus of elasticity of current luting cements are far lower than that of posts and dentine, which may create a zone of high stresses especially when a thick layer of cement is present in a wide or flared canal, leading to inefficient bonding (Boschian Pest *et al.* 2002; Teixeira *et al.* 2009).

More recently, dual cured resin composite materials with different viscosities have been used in combination with fibre posts to restore structurally compromised ETT (Aksornmuang *et al.* 2014). Most of these materials are methacrylate resin based with high filler content and superior mechanical properties than those of resin cements. Previous studies have shown that incorporation of high amounts of filler improve the rigidity of the luting agent but increase stress development during polymerisation, which in turn affects the integrity of adhesive interface, reducing bond strength and increasing microleakage (Condon and Ferracane 2000; Ferrari *et al.* 2009). The higher viscosity that is associated with higher filler load (Lee *et al.* 2006) also impedes the injection of the material into the root canal producing gaps and voids that may provide a site for recurrent caries to develop. The composition of the matrix (Ellakwa *et al.* 2007) also has an effect on both viscoelastic and rheological properties, which influence the contraction

stress and microleakage of the direct restoration (Tay *et al.* 2005; da Silva *et al.* 2007). Consequently, the incorporation of low molecular weight monomers within methacrylate resin composite materials can enhance the flexural properties and lower viscosity (Davidson and Feilzer 1997).

On the other hand, numerous efforts have been made recently on the development of new monomers to be added into the formulation of dental resin composites with the aim of improving their functionality, quality and durability. Several low viscosity ionic mono and dimethacrylate monomers containing quaternary ammoniums groups such as 1,2-Methacryloyloxydodecylpridinium bromide (MDPB) and bis (2-methacryloyloxyethyl) dimethylammonium bromide (IDMA) imparting antimicrobial properties in conjunction with existing dental dimethacrylate-based monomers have been reported (Imazato *et al.* 2003; Antonucci *et al.* 2012). However, adverse effects on mechanical properties associated with high monomethacrylate content were found. In addition, some of the quaternary ammonium based monomers exhibit miscibility problems with hydrophobic dimethacrylates (Antonucci *et al.* 2012).

Eugenyl methacrylate monomer (EgMA), a low molecular weight monomer obtained by modifying the chemical structure of eugenol was reported by Rojo et al (Rojo *et al.* 2006), which has a polymerisable methacrylate group that allows facile free radical polymerisation reaction while impart desired functionalities (Rojo *et al.* 2006). Furthermore, previous studies on rheological properties of the EgMA copolymers confirmed the formation of branching structures with a range of degree of crosslinking that were responsible for the elastic or viscoelastic properties of these systems. In addition, this monomer also demonstrated intrinsically bactericidal properties against different microorganisms including *Streptococcus mutans* (Rojo *et al.* 2008a), which is involved in composite failures associated with secondary caries (Mjör *et al.* 2000; Sarrett 2007).

The purpose of this study was to formulate and characterise new dual cure resin composite materials based on EgMA monomer and Bis-GMA/TEGDMA resin systems for endodontic post cementation and core build-up restoration. The addition of this monomer was expected to enhance the viscoelastic properties, the mechanical response of the composites and potentially impart some antibacterial property to the resin system by virtue of the EgMA residues (Rojo *et al.* 2008a). The influence of this monomer on curing kinetics, viscosity, physical and mechanical properties of the experimental composites are reported and the results compared with those of a commercially available dual cured resin composite core material.

4.1.2 Materials and Methods

4.1.2.1 Materials

2, 2-Bis [4- (2-hydroxy-3 methacryloyloxypropyl)-phenyl] propane (Bis-GMA) and tri-ethylene glycol dimethacrylate (TEGDMA) were purchased from Esschem Europe Ltd. (Durham, UK). Benzoyl peroxide (BPO) and A-174 silane coupling agent (3-trimethoxysilyl propylmethacrylate) were supplied by Merck (Frankfurt, Germany). Methacryloyl chloride (95%) was purchased from Alfa Aesar, UK. Camphoroquinone (CQ), N, N - dimethyl-p-toluidine (DMpT), eugenol and trimethylamine were purchased from Sigma-Aldrich, Company Ltd., Dorset, UK. The fillers used in this study were hydroxyapatite (HA, Plasma Biotal Ltd., Tideswell, Derbyshire, UK) and zirconium oxide (ZrO_2, Fisher Scientific Ltd., Loughborough, UK) with a mean particle size diameter of 3-5 µm and 18 µm respectively, which were silanised according to the method described elsewhere (Deb *et al.* 1996). Solvents used were of HPLC grade from Acros-Organics UK. All other reagents were purchased from Sigma Aldrich and used as received, except BPO that was purified by fractional crystallisation from ethanol. A commercially available resin composite material (Clearfil™DC Core plus, Kuraray, Tokyo, Japan) was used as a commercial reference.

4.1.2.2 Synthesis and characterisation of Eugenyl Methacrylate

EgMA monomer (MW = 232.23 g/mol) was synthesised as reported previously by Rojo *et al.* (2006). In brief, eugenol (0.061 mol) and triethylamine (0.061 mol) were dissolved in 50 ml of dichloromethane. Methacryloyl chloride (0.076 mol) was dissolved in 10 mL of dichloromethane and then added drop wise whilst the reaction mixture was kept in an ice bath under magnetic stirring for 48 hours. The triethylamine chlorhydrate formed was then removed by filtration and the mixture washed with NaOH (5% w/v), neutralised with saturated NaCl and subsequently dried over anhydrous $MgSO_4$. The solvent was then filtered and removed under reduced pressure and the product purified by flash chromatography using a mixture of ethyl acetate / hexane (10/90 v/v) as an eluent. The EgMA monomer was characterised by ATR-FTIR (ATR-Perkin-Elmer-Spectrum One) and ^{1}H-NMR (Bruker-300 MHz) spectroscopies. The FTIR spectrum was recorded in the 4000 cm^{-1} to 650 cm^{-1} region with a wavenumber step of 0.5 cm^{-1}. ^{1}H NMR spectra were recorded at 25 °C and deuterated chloroform was used as a solvent.

4.1.2.3 Preparation of composites

Three different experimental composites namely BTEg0, BTEg5 and BTEg10 were prepared and their respective composition is listed in Table 4.1-1. Briefly, a batch of monomer mixture was first prepared and divided in two separate pastes and the initiator and activator were added respectively to avoid self-polymerisation. Then the corresponding amount of silanised fillers was added to each paste and mixed by magnetically stirring for 24 h. After complete wetting of the fillers, the pastes were sheared with a Teflon spatula against a glass slab surface in a dark room to ensure thorough dispersion of fillers in the resin. Subsequently, equal masses of the two pastes were hand-mixed using a stainless steel spatula for 30 seconds and carefully placed into different moulds avoiding bubble entrapment. The upper and lower surface of the mould was covered with glass slides and then cured by visible light for 40 s each side by overlapping, using Optilux 501 (Demetron, Danbury, USA) dental curing unit with an irradiance of 400 ± 50 mW cm^{-2}. The Clearfil™DC commercial reference was mixed

according to the manufacturer's instructions, moulded and cured by the same procedure described above.

Table 4.1-1 Formulation codes and composition (% w/w) of experimental composites

Composites	Monomers (wt.%)			Fillers (by wt.%)
Name	BisGMA	TEGDMA	EgMA	HA/ ZrO_2 (4:3 wt:wt)
BTEg0	17.5	17.5	0	65
BTEg5	15.0	15.0	5	65
BTEg10	12.5	12.5	10	65

The initiators (0.5% benzoyl peroxide + 0.5% camphorquinone) and activator (N,N dimethyl p-toluidine 1:1 molar ratio) were added as wt.% with respect to monomer of the final resin monomers blend (100 wt.%) formulation

4.1.2.4 Viscosity of the uncured composites

The viscosity of the experimental composite pastes were determined at 25 °C at different shear rates using a digital viscometer (Brookfield DV-E; Middleboro, USA) with a SC4-14/6R spindle configuration and ± 0.1% accuracy. The viscosity value for each paste (2.1 ml) is reported in milliPascal/second (mPa s) for a 2 min time span; with the measurement repeated twice for each composite.

4.1.2.5 Degree of conversion

In order to assess the degree of cure of the composites, FTIR spectra of the resins were recorded before and after cure using a FTIR spectrometer with an ATR attachment (Perkin-Elmer, USA). Spectra were obtained over 4000–650 cm^{-1} region and acquired with a resolution of 4 cm^{-1} and a total of 16 scans per spectrum. The spectra of the polymer were obtained by curing a small amount of each composite between two translucent Mylar strips, which were pressed to produce a very thin film. Three cured specimens of each group were tested 10 minutes after curing and after 24 h storage at 37 °C. The degree of cure was then determined using the Eq. (1)

$$Degree\ of\ conversion\ (\%) = \left[1 - \frac{\left({}^{A_{1637}}/_{A_{1608}}\right)_{polymer}}{\left({}^{A_{1637}}/_{A_{1608}}\right)_{monomer}}\right] \times 100 \quad (1)$$

where A_{1637} and A_{1608} correspond to the absorbance of the aliphatic $\upsilon_{C=C}$ and aromatic $\upsilon_{C=C}$ peaks registered at 1637 cm^{-1} and 1608 cm^{-1} respectively before and after polymerisation.

4.1.2.6 Curing parameters

The depth of cure and maximum curing temperature of the resin composites were determined in accordance with the respective standard ISO 4049 and ISO 5833 techniques. Briefly, composite materials (n=3) were filled in a white Teflon mould with a cylindrical cavity of 15 mm height and 4 mm diameter while the top of the mould was covered with a transparent polymer strip. The specimens were then polymerised for 40 s from the top side. Immediately after irradiation and removal from the mould, the un-polymerised parts were scraped off with a plastic spatula. Subsequently, the depth of the cured material was measured with a digital calliper (DURATOOL, UK) in three places and an average was obtained. The absolute length was divided by two; the average of three measurements was then reported as the depth of cure.

A thermocouple (1.3 mm diameter) fitted to a high-sensitivity temperature recorder (KM1242, Herts, UK) was used to measure the polymerisation exotherm. The wire was placed centrally in a cylindrical Teflon mould filled with each material and its stripped ends were levelled with the surface to be irradiated. The materials were polymerised for 40 s with an irradiance of 400 ± 50 mW cm^{-2} from one side and the maximum temperature was reported during the polymerisation cycle. Three measurements were done for each material at room temperature.

4.1.2.7 Thermal properties

Glass transition temperature (*T*g) of the experimental composites was measured on a Differential Scanning Calorimetry (DSC, Perkin Elmer) and determined as the midpoint

of the heat capacity transition registered. Samples of approximately 10 mg were introduced in the aluminium pans and heated from 0 °C to 230 °C at the rate of 20 °C/min in an inert N_2 atmosphere and two repeats were performed for each composite.

4.1.2.8 Dynamic mechanical analysis (DMA)

Storage (*E'*) and loss modulus (*E''*) as function of temperature were determined for the cured composites using Dynamic Mechanical Analysis (DMA) (Perkin-Elmer, DM8000). A frequency of 1 Hz was applied and a temperature range between 25 °C and 185 °C (heating rate of 2 °C/min) was selected. Rectangular specimens were fabricated for DMA test by filling a Teflon mould (2 mm x 2 mm x 40 mm) with unpolymerised material, which was then light cured with an irradiance of 400 ± 50 mW cm^{-2} following the same procedure mentioned earlier. Three samples for each group were tested and mean values are reported.

4.1.2.9 Mechanical properties

4.1.2.9.1 Flexural properties

Three-point bending test was carried out according to the ISO 4049 (ISO4049) using a universal testing machine (Instron model 5569A-Series Dual Column, High Wycombe, UK) at a cross-head speed of 1 mm/min with span length fixed at 20 mm. Six specimens with dimensions (2 mm x 2 mm x 25 mm) were fabricated using a Teflon mould and tested after 24 h of storage at 37 °C. Flexural strength (σ) and flexural modulus (*E*) were calculated using Eq. (2) and (3) respectively.

$$\sigma = \frac{3FL}{2bh^2} \quad (2)$$

$$E = \frac{L^3}{4bh^3} \times \frac{F}{Y} \quad (3)$$

where *F* = maximum strength, *L* = distance between the rests, *b* = width of the specimen, *h* = height of the specimen, and *F/Y* = slope of the linear part of the stress-strain curve.

4.1.2.9.2 Compressive strength

Six cylindrical specimens (6 x 4 mm) were prepared and tested after 24 h maintained dry at 37 °C using a universal testing machine at a cross-head speed of 1 mm/min. Compressive strength (*S*) was calculated using Eq. (4)

$$S = \frac{F}{(d/2)^2 \times \pi} \quad (4)$$

where, *F* = maximum strength and *d* = diameter of the specimen.

4.1.2.9.3 Microhardness test

Knoop microhardness measurements were carried out on the composite samples (n = 6) with a Durimet microhardness tester (Leitz,Wetzlar, Germany) using a load of 100 g for 15 s. Three measurements were made per sample and the mean values are reported.

4.1.2.10 Fracture surface examination

The fractured surfaces of the three-point bend test specimens were analysed using Scanning Electron Microscopy (SEM, Hitachi High Technologies, S-3500N) at an accelerating voltage of 10 KeV and magnifications 2000x and 5000x. The fracture surface of tested samples was coated with gold using sputter coater before analysis.

4.1.2.11 Radiopacity

Five discs (15 mm diameter × 1 mm thick) were prepared from each composite group and digitally photographed alongside a high purity aluminium step-wedge (1100 alloy) with thickness varying from 1 to 10 mm with increments of 1 mm as a reference according to ISO 4049 (ISO4049). The images were taken using dental X-ray unit (Heliodent; Sirona, Bensheim, Germany) operating at 70 kV, 8 mA, and 0.2 s) with phosphor plate system (Digora® Optime; Soredex, Tuusula, Finland) to get a radiograph. The radiopacity of the experimental material was determined by comparison with the opacity of aluminum step-wedge. A free image editing software (ImageJ processing and analysis in java, version 1.47v) was used to measure the grey value of the sample and aluminium in the resulting images.

4.1.2.12 Statistical analysis

A one-way (ANOVA) and Tukey's post-hoc test were employed for the statistical evaluation of the data at a level of significance $P<0.05$. Values that were significantly different with respect to the control formulation (BTEg0) and the commercial (Clearfil™DC Core) material are marked with one asterisk (*) or two asterisks (**) respectively in the corresponding results.

4.1.3 Results

4.1.3.1 Synthesis and characterisation of Eugenyl Methacrylate

Eugenol methacrylate was synthesised by reacting eugenol with methacryloyl chloride, using triethylamine as a catalyst with a yield of 80%. The FTIR spectra of EgMA is shown in Fig. 4.1-1 and the absorption bands arising at 1725 cm^{-1} (C=O carbonyl stretching vibration) and 1637 cm^{-1} (C=C in the acrylic and allyl groups) confirm the conversion of eugenol to eugenyl methacrylate. The stretching frequency at 1608 cm^{-1}(C=C aromatic) is due to the unsaturated aromatic ring, the peak at 1434 cm^{-1} can be attributed to CH_2= in the allyl group and the strong peak at 1120 cm^{-1} assigned to C–O stretching from ethers.

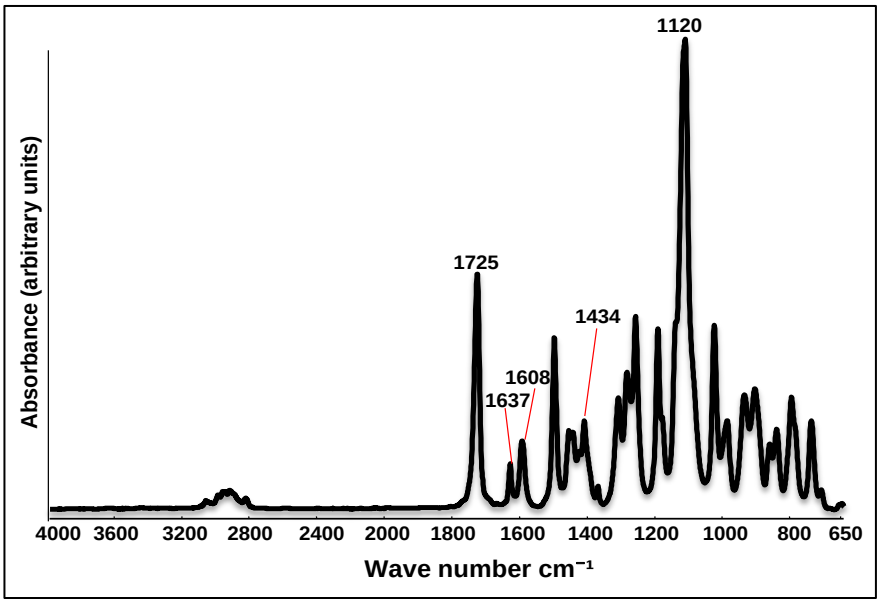

Figure 4.1-1 ATR-FTIR spectrum of EgMA monomer. Vibrational peak assignments: $\upsilon_{C=O}$ 1725 cm^{-1}; $\upsilon_{C=C,\ Acryl}$ & $\upsilon_{C=C,\ Allyl}$ 1637 cm^{-1}; υ_{Ar} 1608 cm^{-1}; $\upsilon_{C-H,\ Allyl}$ 1434 cm^{-1} and $\upsilon_{C-O,\ Ether}$ 1120 cm^{-1}

The molecular formula and ^{1}H-NMR spectrum of the monomer are shown in Fig. 4.1-2 The peak assignments δ (ppm) are as follows: δ_H 7.0 (H_5-Ar), 6.8 ($H_{3,6}$-Ar), 6.4 and 5.7 (CH_2^{β} =C), 6.0 - 5.8 (C*H*=CH_2), 5.1 (CH=CH_2), 3.8 (CH_3 - OPh), 3.4 (CH_2Ph), 2.1 (CH_3^{α}).

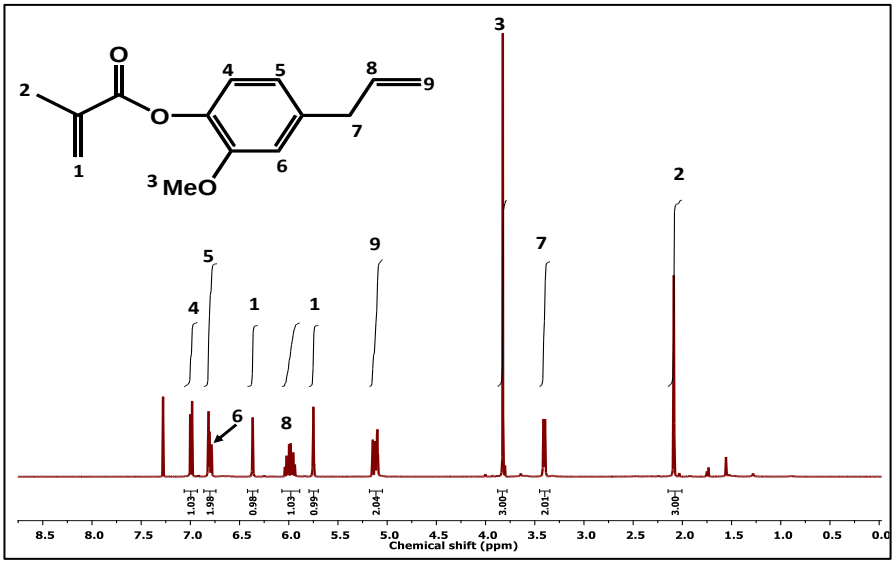

Figure 4.1-2 Assignment and ^{1}H-NMR spectrum of EgMA monomer in $CDCl_3$. Normalised integral values are displayed beneath the corresponding peaks

4.1.3.2 Viscosity

The experimental composite materials exhibited a decreasing viscosity with an increasing shear rate as shown in Fig. 4.1-3. This pseudoplastic or shear thinning behaviour was more pronounced with increasing content of EgMA monomer in the formulations.

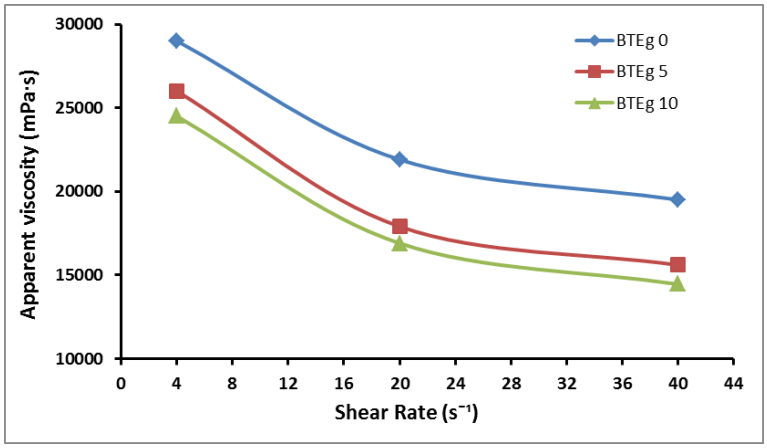

Figure 4.1-3 The apparent viscosity of uncured experimental composites at different shear rates

4.1.3.3 Curing parameters

The degree of conversion, curing depth and polymerisation exotherm of all materials tested in this study are shown in Table 4.1-2. The degree of conversion for each formulation was calculated using Eq. (1) and the values ranged between 64 to 72% (Table 4.1-2). Incorporation of EgMA monomer at 10% into composites formulation decreased the degree of conversion initially, however, there were no significant difference in DC between composites containing EgMA and the control at 24 h post curing. The experimental composites exhibited comparable depths of cure among them but their values were significantly higher in comparison to the commercial composite (P<0.05). The inclusion of EgMA in the formulation also lowered the polymerisation exotherm with increasing concentration at 10% (Table 4.1-2).

Table 4.1-2 The degree of conversion (10 minutes and after 24 h post curing), depth of cure and polymerisation exotherm of the experimental composites and commercial material [mean (SD), n=3]

Composites	DC in % 10 min post cure (SD)	DC in % 24 h storage at 37 °C (SD)	Depth of cure in mm (SD)	Polymerisation exotherm °C (SD)
BTEg0	**72 (1.0)**	**73 (2.0)**	**4.9 (0.2)****	**35.5 (0.2) ****
BTEg5	**68 (1.0) ****	**71 (1.0) ****	**4.7 (0.5)****	**34.9 (0.3) ****
BTEg10	**64 (1.0) *,****	**70 (2.0) ****	**4.2 (0.2)****	**31.4 (0.4) *,****
Clearfil™DC	**72 (2.0)**	**75 (1.0)**	**2.2 (0.1)**	**37.1 (0.3)**

*** Differences were statistically significant with respect to control BTEg 0 composite (P<0.05)**
**** Differences were statistically significant with respect to commercial Clearfil DC Core material (P< 0.05)**

4.1.3.4 Thermal analysis

The DSC thermograms for the experimental composites are shown in Fig. 4.1-4. Composites containing EgMA monomer exhibited Tg values ranging between 106 ± 4.2 °C and 114 ± 3.5 °C which were slightly lower than that of the composite without EgMA (119 ± 2.1 °C). However, no significant differences in Tg were found between experimental composites (P>0.05).

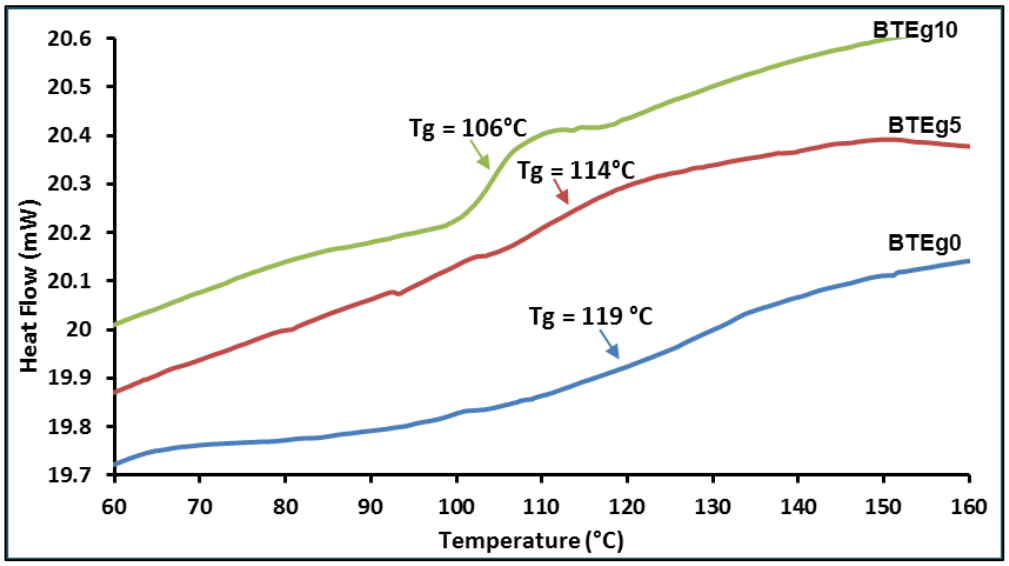

Figure 4.1-4 DSC representative curves of the experimental composites.

4.1.3.5 Dynamic mechanical analysis (DMA)

Fig. 4.1-5 illustrates the evolution of Tan δ with the temperature for experimental composites and the commercial material.

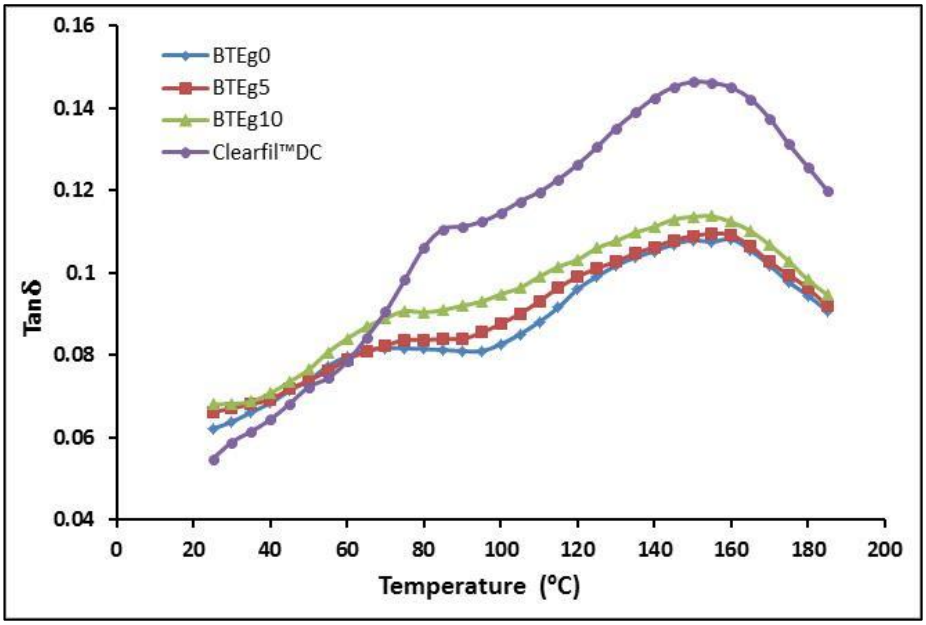

Figure 4.1-5 DMA curves for Tan δ of the experimental composites and commercial material

The mean values of storage modulus (*E'*), loss modulus (*E''*) and damping factor (Tan *δ*) at 37 °C were extracted from DMA curves and presented in Table 4.1-3. There were no statistically significant differences (*P*>0.05) in the *E''* and Tan *δ* due to the presence of EgMA in the experimental composites while the storage modulus of 10% EgMA formulation was significantly lower than the control. At 37 °C the commercial composite showed statistically significant lower Tan *δ* value than the experimental composites while at higher temperatures Clearfil™DC Core exhibited a higher Tan *δ* peak.

Table 4.1-3 Dynamic mechanical properties of the experimental composites and commercial material at 37 °C, 24 h after curing [mean (SD), n=3]

Composites	*E'* in GPa (SD)	*E''* in GPa (SD)	Tan *δ*×10^3 (SD)
BTEg0	**120.2 (2.9)**	**7.9 (0.4)**	**66.5 (1.7)****
BTEg5	**114.1 (2.5)**	**7.8 (0.3)**	**68.0 (1.2)****
BTEg10	**104.7 (4.7)*,****	**7.3 (0.2)**	**70.0 (1.4)****
Clearfil™DC	**120.8 (2.1)**	**7.6 (0.2)**	**63.0 (0.6)**

*** Differences were statistically significant with respect to control BTEg 0 composite (*P*<0.05).**

**** Differences were statistically significant with respect to commercial Clearfil DC Core material (*P*<0.05).**

4.1.3.6 Mechanical properties

The results in Table 4.1-4 show that the addition of EgMA into the composite formulations increased both flexural and compressive strength. The statistical analysis showed no significant difference in the flexural modulus of the experimental materials (*P*>0.05), whilst the flexural strength of both EgMA formulations and the compressive strength of 5% EgMA formulation were significantly higher than the control. The microhardness of BTEg10 (10 wt.% EgMA) composites was significantly lower than that of control (*P*<0.05). The mechanical properties of the experimental formulations were comparable to that of the commercial composite except for the microhardness which was significantly lower (*P*<0.001).

Table 4.1-4 Flexural modulus (FM), flexural strength (FS), compressive strength (CS) and Knoop hardness number (KHN) of the experimental composites and commercial material (mean (SD), n = 6)

Composites	FM in GPa (SD)	FS in MPa (SD)	CS in MPa (SD)	KHN (SD)
BTEg0	11.4 (0.9)	95.9 (3.4)	198.2 (20.0)	39.3 (0.6) **
BTEg5	11.0 (0.8)	114.2 (9.4)*	234.6 (17.8)*	38.1 (0.9) **
BTEg10	9.8 (0.5) **	121.9 (11.0)*	214.6 (19.9)	34.0 (0.9) *,**
Clearfil™DC	11.5 (1.3)	110.5 (15.9)	210.9 (29.2)	54.6 (2.2)

*** Differences were statistically significant with respect to control BTEg 0 composite (*P*<0.05)**
**** Differences were statistically significant with respect to commercial Clearfil DC Core material (*P*<0.05)**

4.1.3.7 SEM of the fracture surface of the composites

Representative SEM images of the fractured surfaces of the flexural test specimens from each of the three experimental groups are shown in Fig. 4.1-6. Variance was detected between the control and the remaining experimental groups. The incorporation of EgMA monomer resulted in less fillers agglomeration and the particles were well dispersed into the resin matrix.

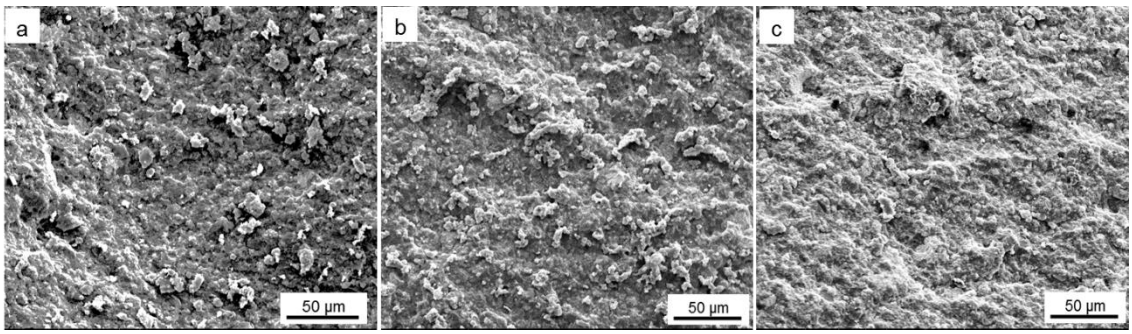

Figure 4.1-6 Representative SEM images of three point bending fracture surfaces of experimental composites (at 2000x magnification). (a) BTEg0 control, (b) BTEg5 and (c) BTEg10

4.1.3.8 Radiopacity

Fig. 4.1-7 shows the radiopacity of the experimental and commercial composites with the grey-scale value of aluminium step wedge measurements in (mm AL). All the composites exhibited a favourable radiodensity values around 3 mm Al that satisfy the ISO 4049 specifications and were significantly higher than that of commercial composites (P<0.05). The radiopacity of BTEg10 composites was significantly higher than that of control (BTEg0) at P<0.05.

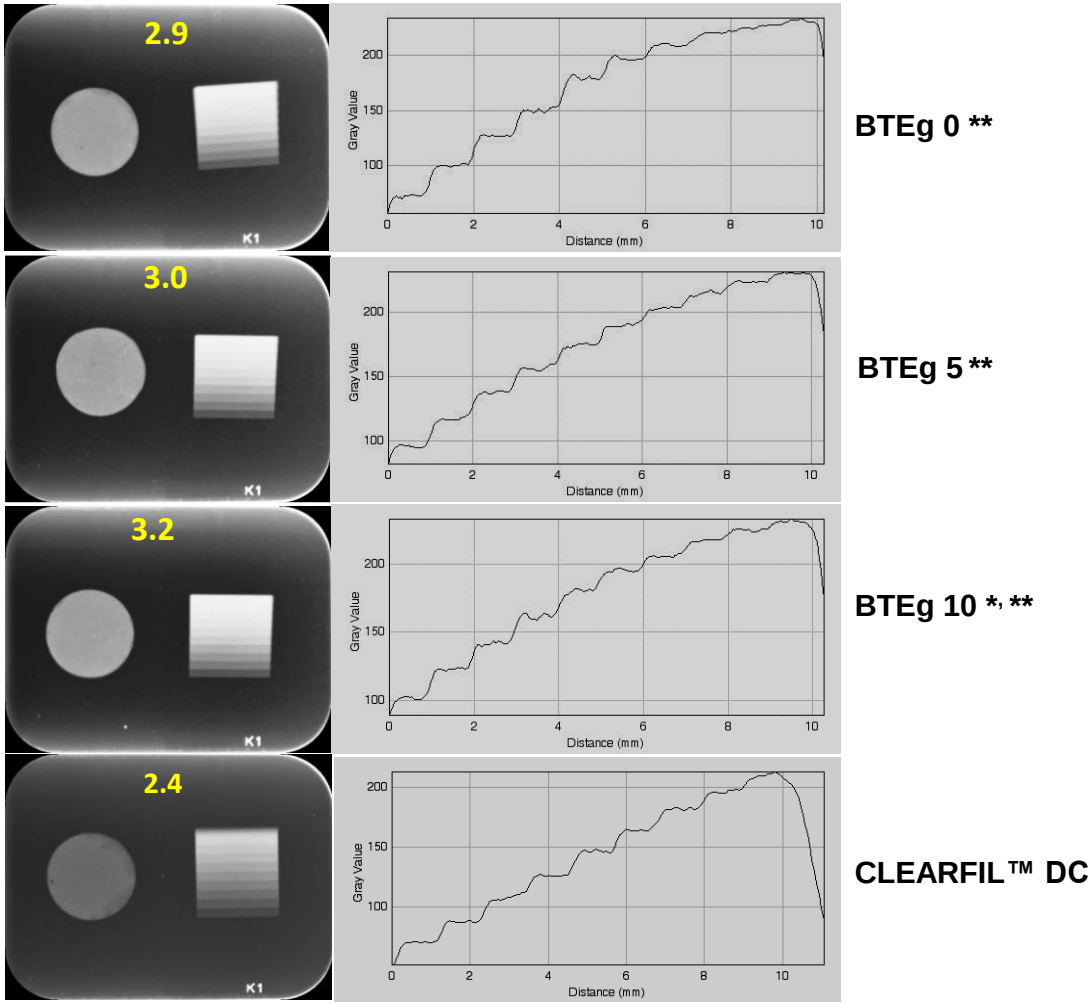

Figure 4.1-7 Representative radiographs of experimental composites and Clearfil™DC Core material in relation to the density of the aluminium step wedge (n=5)

4.1.4 Discussion

New dental resin composite with methacrylate derivative of eugenol was developed for intracanal post cementation and core build-up. The incorporation of this derivative provided an improvement in the initial handling viscosity, polymerisation exotherm, mechanical strength, viscoelasticity and radiopacity, while slightly reduced the initial degree of cure and *T*g. A significant reduction in storage modulus and microhardness was also observed at higher concentration.

Eugenol methacrylate monomer was synthesised by a typical acylation reaction as described earlier by Rojo et al (Rojo *et al.* 2006). The modification of the chemical structure of eugenol through the phenolic group allows it to participate in polymerisation reactions rather than to inhibit them. The EgMA monomer showed typical peaks arising due to the carbonyl and the allyl group in the FTIR spectrum that confirmed the methacrylation of eugenol. The ^{1}H NMR spectra showed the resonance signal corresponding to the proposed structure illustrated in Fig. 4.1-2 and the absence of the characteristic phenol peaks at d $\delta_{1H\text{-}NMR}$ 5.5 ppm confirmed the reaction of eugenol.

The miscibility of comonomers within a polymerisable mix is important to overcome phase separation post polymerisation. The monomer EgMA exhibited complete miscibility with Bis-GMA/TEGDMA mixtures and there was no evidence of phase separation. The composition of the experimental composites were based on a set of design of experiments with monomers Bis-GMA used for imparting stiffness and lower shrinkage, TEGDMA to lower viscosity and allow crosslinking and EgMA as a polymerisable monomer derivative of eugenol. The selection of EgMA contents within the experimental formulations was based on previous studies which demonstrated that 10 wt.% of EgMA is enough to impart the mechanical and biological benefits of the eugenol residues with absence of cyto or genotoxic effects. The cytotoxicity of the monomer has been reported earlier and was comparable to those observed for other monomers commonly used to prepare analogue restorative dental materials (Rojo *et al.*

2006). Furthermore, the cytocompatibility of EgMA containing polymer matrices have also been reported previously (Rojo *et al.* 2008b) showing the absence of residual monomers and very good cytocompatibility.

The low viscosity of the EgMA monomer also functioned as an excellent diluent for Bis-GMA and enhanced the initial handling viscosity allowing easy dispersion of the fillers within the resin matrix. This is a common finding for most organic fluids, which may be attributed to temporary deformation and alignment of the flexible molecules in the streamlines of increasing flow. A low viscosity of these composites during the working period is also desirable as it can facilitate their injection into the root canal through the delivery devices, and for effective impregnation of dentinal substrates and post surface (Finger *et al.* 1994). It also reduces the polymerisation shrinkage stress within the material during early setting (Davidson and De Gee 1984; Labella *et al.* 1999), and the stress relief via resin flow relaxation can reduce the possibility of gap formation (da Silva *et al.* 2007) and marginal leakage (Swift Jr *et al.* 1996; Montes *et al.* 2002), which can enhance the longevity of the restorations. It has also been reported that the lower viscosity resin composites provide significantly higher adhesion to post surface attributed to higher intrusion on the substrates (Aksornmuang *et al.* 2014).

The composite formulations with 65% by weight of fillers were used for all formulating composites based on flowability. The selection of the fillers used in the composite formulation stemmed from the excellent established biocompatibility of these fillers, the ability to enhance the modulus of polymers, in addition hydroxyapatite being similar to the mineral component of dentine and ZrO_2 functioning as a radiopacifying agent (Zhang and Darvell 2010; Alhashimi *et al.* 2012). The weight fractions of the fillers were selected on the basis of optimal properties and radiographic appearance. Moreover, the homogenous distribution and the stable dispersion of the fillers without any sedimentation, demonstrated the stability of the composite mixtures at the different storage periods. The experimental composite was designed as dual-cured resin system with better clinical handling as it allows extended working time and secures

polymerisation in deep parts of the canal (Hofmann *et al.* 2001; Arrais *et al.* 2008; Kournetas *et al.* 2011).

The degree of cure were found to be above the minimum acceptable values for clinical use (>55%) (Silikas *et al.* 2000) that were comparable with other dual-cured dimethacrylate based composites (Franz *et al.* 2014). The significant reduction in the degree of conversion observed 10 min post curing (Table 4.1-2) of composites containing 10% EgMA with respect to control is the consequence of the bi-functional nature of acrylic and allylic double bonds in the EgMA moiety, which further confirms the participation of the monomer during the bulk polymerisation (Rojo *et al.* 2009), leading to either branching or crosslinked structures with unreacted allylic bonds from the pendant eugenyl moiety. However, the degree of cure increased when measured at 24 h with values comparable to formulations without EgMA indicating post-curing. Nevertheless, it is important to highlight that the post curing polymerisation observed was very limited, indicating that most of post-irradiation polymerisation occurred in the first few minutes after light exposure (Pilo and Cardash 1992; Tarumi *et al.* 1999).

The curing depth values of the experimental composites exhibited statistically significant higher values in comparison to the commercial composite. This difference was associated with composition, catalyst type and concentration and also transmission coefficient which depend on the shade of the resin (Kawaguchi *et al.* 1994). Curing depth depends on the type and concentration of initiator used, irradiation conditions and material composition (including opacity) (Rueggeberg *et al.* 1997; Leprince *et al.* 2013). Here, the use of the same irradiation conditions and the same concentration of photoinitiator (0.5 wt.% of resin mixture) in the formulation of dual-cure composites resulted in statistically similar curing depths of the experimental composites. However, the progressive reduction with the content of EgMA was attributed to the increase of opacity of these composites that reduce the light transmittance (Mongkolnam and Tyas 1994).

The addition of EgMA decreased the maximum curing temperature; being more pronounced for BTEg10 formulation, which exhibited a peak temperature of 31.4 °C and was significantly lower than the control and commercial material. This reduction in the exothermic polymerisation of the composite material without compromising the mechanical properties constitutes an additional advantage preventing thermal damage on adjacent root dentine whilst the curing occurs within the endodontic cavity.

The glass transition temperature of the composites containing EgMA exhibited lower *T*g, which can be attributed to the lower *T*g of EgMA homopolymer (about 95 °C) (Rojo *et al.* 2006) in comparison with the other methacrylate derivatives in the composite (Bis-GMA and TEGDMA). However, all the experimental composites post curing showed *T*g values much higher than the oral cavity would normally be exposed to, thus ensuring no softening or hardening during clinical function.

DMA was used to measure the viscoelastic properties at a frequency and temperature range experienced in the oral cavity. The experimental composites containing 10% EgMA (BTEg10) exhibited a significantly lower *E'* due to the molecular flexibility by virtue of the higher EgMA component, however this effect was not observed in the composites containing 5% EgMA (BTEg5) due to the lower concentration. As the temperature was increased, the tan *δ* remained constant for all experimental composites indicating higher damping and thermal stability. This is attributed to the silanation of the fillers that enhances the interfacial adhesion with the matrix in addition to the formation of a slightly cross-linked network in composites containing EgMA. Clearfil™ DC Core, the commercial material was tested under identical conditions, showed the lowest value for tan *δ* and the highest *E'* indicating the stiffness of these materials as consequence of the filler higher content (74 wt.%). Therefore, in accordance with previous studies, the viscoelastic properties of experimental composites offer a considerable advantage over the commercial composite material with respect to polymer network's ability to relieve the shrinkage stress through chain viscoelastic relaxation at normal oral temperature

(Charton *et al.* 2007) and to their thermal stability at the more challenging environment of the oral cavity and therefore rendering better luting ability (Sterzenbach *et al.* 2012).

The static mechanical tests results showed that the EgMA monomer increased the resistance of composites against bending and compression stresses. This is consistent with the results obtained from DMA which confirm the effect of EgMA, which allows for both crosslinking and forming branched structures. The crosslinked networks arising due to TEGDMA and EgMA from both EgMA lead to strengthening whilst an increase in ductility is observed due to the branching. However, it is evident that an optimum concentration of EgMA results in an increase of both compressive and flexural strength. It has been shown in previous studies that increasing monomer concentration beyond certain limits does not lead to further improvement in mechanical properties (Rojo *et al.* 2008b; Rojo *et al.* 2009). However, the mean values for flexural strength and compressive strength of eugenyl containing composites were similar to that of the commercial material ($P>0.05$). The KHN of Clearfil DC Core material was significantly higher than that of the experimental composites. The hardness of composite resins reflects their molecular chain flexibility and degree of polymerisation (Ferracane 1985; Furuse *et al.* 2011) and is affected by other factors such as resin matrix type, filler type and filler load (Kim *et al.* 2002; Ruttermann *et al.* 2010). Not only is the filler content in Clearfil DC Core is much higher, the type of filler is different than that of the experimental composites, which may account for lower microhardness. The Knoop hardness of BTEg10 was statistically lower than BTEg5 and BTEg0 ($P<0.05$), however, the values obtained were in the range accepted for clinical applications (O'Brien 1997).

Scanning electron micrographs of the fracture surfaces revealed homogenous distribution of the filler particles and good adhesion between the matrix and the filler in the experimental composites. In particular, EgMA containing composites appeared to show less debonding of the filler particles, which were also smeared by the matrix in comparison with the control (Fig. 4.1-6). These findings suggest a better adhesion and

diffusion of the monomer between the particles that provides a uniform distribution and homogenous matrix (Yuan *et al.* 2007).

Radiopacity of luting resin composites and core materials is important for the clinician for accurate placement and follow up. According to ISO 4049 specifications, the radiopacity of dental composite material should be higher than, or at least equal to, that of the same aluminium thickness which is close to that of dentine. The radiopacity values of the experimental composites were significantly higher than that of commercial Clearfi™ DC Core material and superior to those of enamel and dentine reported in previous studies which range between 1 and 2 mm Al respectively (Attar *et al.* 2003; Hitij and Fidler 2013). The higher radiocontrast of the experimental composites is likely due to the incorporation of zirconia and hydroxyapatite. The addition of EgMA monomer enhanced the filler dispersion within the resin matrix resulting in homogenous mixture which had a significant effect on radiopacity of BTEg 10 composite.

Further studies *in vitro* are needed to evaluate the performance of these composites in restoring structurally compromised ETT in term of bonding ability and reinforcement.

4.1.5 Conclusions

Resin composite containing eugenol methacrylate derivative EgMA was obtained by dual polymerisation mechanism that exhibited outstanding properties in term of handling viscosity, flexural strength, viscoelasticity and radiopacity as potential new materials for post cementation and core build-up restoration in structurally compromised ETT with remarkable enhanced features in comparison with currently used dental composite materials. Therefore, they constitute a novel approach to include these resins with the biological benefits of eugenol that until now has been considered to be incompatible with *in situ* polymerising dental resin composites.

4.2 Influence of a polymerizable eugenol derivative on the antibacterial activity and wettability of a resin composite for intracanal post cementation and core build-up restoration

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***Dental Materials* 2016, 32 (7): 929-939**

Authors' contribution

I carried out the experimental work, interpreted data, designed and wrote the manuscript of the paper. Niazi S.A participated in microbiological testing. All authors helped to evaluate and edit the manuscript.

Abstract

Objectives: Eugenol has been used in dentistry due to its ability to inhibit the growth of a range of microorganisms, including facultative anaerobes commonly isolated from infected root canals. The aim of this study was to evaluate the antibacterial activity of the experimental composites containing eugenyl methacrylate monomer (EgMA), a polymeric derivative of eugenol, against a range of oral bacteria, commonly associated with failure of coronal and endodontic restorations. *In vitro* composite behaviour and wettability were also studied in conjunction with their antibacterial activity.

Methods: EgMA monomer (5 and 10% by weight) was added into BisGMA/TEGDMA resin based formulations with filler mixtures of hydroxyapatite (HA) and zirconium oxide ZrO_2. The antibacterial activity of the experimental composites against *Enterococcus faecalis, Streptococcus mutans and Propionibacterium acnes* were evaluated by direct contact test and compared with composite formulation without inclusion of EgMA. To clarify the antibacterial mode of action, agar diffusion test (ADT) was also performed. Water sorption, solubility, diffusion coefficient, contact angle and surface free energy as complementary clinically relevant properties were determined.

Results: Water sorption and wettability studies showed reduction of water uptake and surface free energy values with increasing content of EgMA monomer, resulting in significant increase in the hydrophobicity of the composites. No inhibition zones were detected in any of the composites tested against the three bacteria employed as expected, due to the absence of any leachable antibacterial agent. The covalently anchored EgMA monomer with the composite surface exhibited an effective bacteriostatic activity by reducing the number of CFUs of the three species of bacteria tested with no significant dependence on the concentration of EgMA at 5 and 10% by weight. The surface antibacterial activity *R* of the experimental composites were different against the three tested species with values in the range 2.7–6.1 following the order *E. faecalis* < *S. mutans* < *P. acnes*.

Significance: The incorporation of EgMA monomer within polymerisable formulations provides a novel approach to yield intrinsically antibacterial resin composites for different dental applications.

4.2.1 Introduction

Endodontically treated teeth (ETT) are more susceptible to fracture than vital teeth due to the significant reduction of tooth tissue as a result of the endodontic and restorative treatment accompanied with changes in chemical composition of dentine due to loss of water and collagen (Rivera and Yamauchi 1993). Although it is well established that coronal coverage significantly improves the clinical success rate of endodontically treated posterior teeth, the choice of restoration depends on the amount of remaining tooth structure and functional requirements.

Composite resins have the advantage of bonding to residual coronal and root canal dentine, which may assist in strengthening the tooth (Boschian Pest *et al.* 2002; Wu *et al.* 2007) and offer an alternative technique for restoration of ETT (Taha *et al.* 2011). Dual cure resin composites core materials with different viscosity are currently used within the canal for fibre posts cementation, to restore the structurally compromised ETT. They have superior mechanical properties than those of resin cements and result in lower stress, reducing the load transfer on the root dentine and surface of the post (Boschian Pest *et al.* 2002).

However, resin composites lack antibacterial properties and result in more plaque accumulation than other restorative materials (Beyth *et al.* 2007). In addition, any microleakage allows for new bacterial invasion, compounded by the fact that it is difficult to completely remove bacteria from the root canal system even after careful cleaning and shaping and the minimally invasive approach during restoration of teeth will possibly maintain more residual bacteria within the dentinal tubules (Farrugia and Camilleri 2015).

There is a rising interest to endow dental restorative materials with sustained antibacterial activity to enhance long term performance (Rojo and Deb 2015), which is expected to lower the risk of reinfection (Vieira *et al.* 2012) and secondary caries (Mjör *et al.* 2000). Different antibacterial agents such as chlorhexidine, fluoride, quaternary ammonium salts and metallic agents (silver, gold and zinc) have been incorporated in

acrylic based composite formulation in order to achieve this goal (Imazato 2003). However, most of these additives cause an adverse effect in terms of mechanical properties, discolouration of the material (Fan *et al.* 2011), toxicity and short-term antibacterial effectiveness (Wilson and Wilson 1993).

Most antibacterial studies reported in literature evaluate the activity of different incorporated antibacterial agents against *Streptococcus mutans*, the main microbial etiological agent of dental caries and the leading cause of resin based composite failure (Miller *et al.* 2015), however other oral microorganisms such as, *Enterococcus faecalis*, *Candida albicans* and *Propionibacterium acnes* are also frequently associated with endodontic infections (Meire *et al.* 2012). *E. faecalis*, in particular, is difficult to remove owing to its considerable virulence factors constituting a source of recurrent infection after conservative as well as surgical treatments (Kayaoglu and Ørstavik 2004). *P. acnes* is an anaerobic Gram-positive bacterium responsible for a wide range of infections and inflammatory conditions (Niazi *et al.* 2010). Therefore, development of antibacterial restorative filling materials to be reliable for a variety of dental applications need a potent antimicrobial agent which acts against a wide range of oral microorganisms.

Eugenol (4-allyl-2-methoxyphenol) is a natural phenolic anti-oxidant essential oil that possesses antifungal activity (Burt 2004) and inhibits the growth of several microorganisms including *Escherichia coli* (Blaszyk and Holley 1998) and facultative anaerobes commonly isolated from infected root canals (Kaplan *et al.* 1999). This compound has been used in combination with zinc oxide in different dental applications such as temporary filling materials and root canal sealers. However, eugenol is not compatible with other methacrylate based restorative materials because of the presence of free eugenol, which interferes with the polymerisation reaction of dental composite resins.

In contrast, eugenyl methacrylate (EgMA) an eugenol derivative (Rojo *et al.* 2006) possess in its chemical structure a polymerisable methacrylic group (Fig. 4.2-1) that

allows the monomer to participate in free radical polymerisation reactions whilst maintaining the antibacterial activity of its natural precursor against different Gram-negative and Gram-positive bacterial species (Rojo *et al.* 2008a).

In our previous study, the experimental composites from Bis-GMA/TEGDMA, a commonly used dental resin system and EgMA were formulated with 65% by weight filler phase comprising of HA/ZrO_2 (Almaroof *et al.* 2016a). These composites were tailored to function as an antibacterial restorative material for intracanal posts cementation and core build-up in the restoration of ETT. The influence of EgMA monomer incorporation on curing, physical and mechanical properties of these new formulations showed that these composites were suited for the application.

However, properties such as water sorption and wettability have detrimental effects on the composite material and bacterial adhesion (Liu and Zhao 2005) which are important parameters toward clinical relevance. Hence in this study, the *in vitro* behaviour and antibacterial activity of these EgMA containing resin composites against a range of oral bacteria commonly associated with the failure of coronal and endodontic restorations are reported.

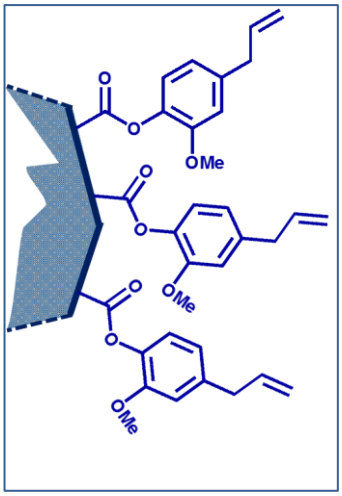

Figure 4.2-1 Proposed example of pendent eugenyl residues (eugenyl methacrylate antibacterial monomer) from the polymer network on the surface of the experimental composite

4.2.2 Materials and Methods

4.2.2.1 Materials and composites formulations

Three batches of dual cure resin composites were prepared by combining 2, 2-Bis [4-(2-hydroxy-3 methacryloyloxypropyl)-phenyl] propane (Bis-GMA) (Esschem Europe, Durham, UK) and tri-ethyleneglycol dimethacrylate (TEGDMA) (Esschem Europe, Durham, UK) in a fixed ratio of 1:1 by weight, representing a total resin phase of 35 wt.% in the formulation. EgMA monomer (MW = 232.23 g/mol.) was synthesised as reported previously (Rojo *et al.* 2006) and added at a level of 0 (reference), 5 and 10 wt.% of the resin phase (Table 4.2-1). All composites were formulated with 65 wt.% filler phase, which contained hydroxyapatite (HA) with a mean particle size diameter of 3-5 µm (Plasma Biotal Ltd., Tideswell, Derbyshire, UK) and ZrO_2 with a mean particle size diameter 18 µm (Fisher Scientific Ltd., Loughborough, UK) in a ratio of 4:3 by weight. The filler particles were silanated with 10% of A-174 (Merck-Frankfurt, Germany) by a wet silanation treatment in 70/30 mix of acetone and distilled water following a method described previously (Deb *et al.* 1996).

Table 4.2-1 Composition of the resin phase of the experimental composites

Composites	**Monomers (in weight percent)**		
Name	**BisGMA**	**TEGDMA**	**EgMA**
BTEg0	**17.5**	**17.5**	**0**
BTEg5	**15.0**	**15.0**	**5**
BTEg10	**12.5**	**12.5**	**10**

All composite formulations contained the same 65 % by weight filler phase of HA/ZrO_2 (4:3, wt/wt).The initiators (0.5% benzoyl peroxide + 0.5% camphorquinone) and activator (N,N dimethyl p-toluidine 1:1 molar ratio) were added as wt.% in respect to monomer at the end of the final resin monomers blend (100 wt.%) formulation

The resin phase was first prepared and divided in two separate portions where initiator system (0.5% benzoyl peroxide (Merck-Frankfurt, Germany) + 0.5% camphorquinone (Sigma-Aldrich, Dorset, UK)) and activator (N,N dimethyl p-toluidine (Sigma-Aldrich, Dorset, UK) 1:1 molar ratio) were added respectively to avoid self-polymerisation. Then the corresponding amount of silanised filler was added to each portion and mixed on a magnetic stirrer for 24h.

4.2.2.2 Sample preparation

Equal masses of the two pastes were hand-mixed using a stainless steel spatula for 30 seconds and carefully placed into Teflon moulds to produce discs of 10 mm diameter and 1 mm thickness avoiding bubble entrapment. The upper and lower surface of the mould was covered with glass slides and then cured by visible light for 40 s each side by overlapping, using Optilux 501 (Demetron, Danbury, USA) dental curing unit performing an irradiance of 400 ± 50 mW cm^{-2}.

4.2.2.3 Water sorption and solubility

Water sorption and solubility were measured according to ISO 4049 (ISO4049). Five disc specimens were prepared for each material. The thickness and diameter of each specimen were measured at 4 and 2 points respectively, using a digital electronic caliper (DURATOOL, UK). Mean values were used to calculate the volume of each specimen in mm^3. The specimens were then placed in a desiccator with anhydrous calcium chloride and maintained at 37 °C. After 22 h, they were removed, stored in another desiccator at 23°C for 2 h and then weighted to an accuracy of ± 0.0001 g using a Mettler-Toledo AG64 balance to obtain the constant initial weight (M_i) and to ensure completion of polymerisation and dehydration. Specimens of each material were immersed in 10 ml distilled water in individual glass containers and then incubated at 37 °C for a total immersion time of 28 days. At noted intervals, the specimens were gently dried on filter paper until free from visible moisture, waved in air for 15 s and weighed 1 min later and returned to the glass containers filled with distilled water. The recorded weight was denoted as the mass of saturated specimen M_s (t, time). Each specimen was then desorbed in a drying oven maintained at 37°C and weighed again until a constant dry mass (M_d) was found. A second absorption–desorption cycle followed to obtain 2nd M_s (t, time) and 2nd M_d in the same way as the first cycle.

Mass change percentage was calculated by the following equations:

$$\text{Sorption mass change percentages} = (M_{s\,(t)} - M_i) / M_i \times 100 \qquad (1)$$

The water sorption (W_{SP}) and solubility (W_{SL}) in µg/mm³ were calculated using the following equations:

$$W_{SP} = M_s - M_d / V \quad (2)$$

$$W_{SL} = M_i - M_d / V \quad (3)$$

where, V is the volume of the sample.

The early stages of diffusion-controlled uptake of water in composites are given by

$$Mt/M^{\infty} = 2\left(\frac{Dt}{\pi l^2}\right)^{1/2} \quad (4)$$

where M*t* is the mass uptake at time *t*, M^{∞} is the equilibrium uptake, *l* is the thickness, and D is the diffusion coefficient.

Diffusion coefficients were evaluated from the slope values of the initial linear part of the sorption curves. A plot of Mt/M^{∞} against $t^{1/2}$ should provide a straight line with the slope, s, then given by

$$s = 2\left(\frac{D}{\pi l^2}\right)^{1/2} \quad (5)$$

and the value of D calculated from the slope value.

$$D = \frac{s^2 \pi\, l^2}{4} \quad (6)$$

4.2.2.4 Measurement of contact angle and surface free energy (SFE)

The contact angle θ and SFE (Y_s) were determined on composites surface discs using the sessile drop method. Ten 5 µl droplets of two liquids with opposite polarity and known surface tension were placed: water (Y_l) 72.8 mN/m^2 and methylene iodide (Y_l) 51.8 mN/m^2.

Y_l refers to the total surface free energy of the liquid. The contact angle was then measured at room temperature 20 s after drop placement by imaging the drop with a magnified digital camera. The profile of the drop was then processed with ImageJ software. The surface free energy (Y_s) of the composites was calculated by the Fowkes' (Fowkes 1964) and Owens' (Owens and Wendt 1969) method.

$$Y_s = Y_s^d + Y_s^P \quad (7)$$

$$(1+\cos\theta) Y_l / 2 = (Y_s^d\ Y_l^d)^{1/2} + (Y_s^p\ Y_l^p)^{1/2} \quad (8)$$

where Y_s^d, Y_s^p, Y_l^d, and Y_l^p are the dispersive and polar components of Y_s of solid and liquid, respectively. The polar and dispersive values for the tested liquids were taken from the literature (Good 1992).

4.2.2.5 Antibacterial assay

4.2.2.5.1 Agar Diffusion Test

Antibacterial activity of the composites against *E. faecalis*, *S. mutans* and *P. acnes* was determined by a standard Agar Diffusion Test (ADT). All discs were sterilised by wiping with 70% ethanol in water and were exposed to UV radiation for 30 mins. The bacteria were evenly spread onto the Fastidious Anaerobic Agar (FAA, Lab M, UK) supplemented with 5% defibrinated horse blood. Under aseptic conditions, 5 discs of each of the test composite (0% EgMA, 5% EgMA, 10% EgMA) were placed onto these FAA plates (one disc for each plate). The plates without discs (n=2) of each bacteria species were used as controls. All plates were incubated anaerobically at 37 °C. The inhibition zones around each specimen were checked after 48 h and again after 4 days.

4.2.2.5.2 Measurement of antibacterial activity on composite surfaces

The antibacterial activity of the composites surface were evaluated *in vitro* against the adherence and growth of *E. faecalis*, *S. mutans* and *P. acnes* following an adapted protocol from ISO 22196:2007 standard for the measurement of antibacterial activity on plastic surfaces (ISO22196).

All discs were sterilised by wiping with 70% ethanol in water and were exposed to UV radiation for 30 min. For each bacterial species, the test was performed on 4 discs of each of the test composites (with 5% EgMA, 10% EgMA) and 8 discs of the control group with no additive (0% EgMA). Half of the control group (4 discs for each bacterial species) were measured for the colony forming units (CFUs) immediately after inoculation and the other half were measured after incubation for 24 h.

The discs were placed in a separate well within a sterile 16-wells plate (Corning®, NY, USA). *E. faecalis, S. mutans and P. acnes* were cultured anaerobically at 37°C overnight on FAA plates (FAA, Lab M, UK) supplemented with 5% defibrinated horse blood. One loopful of the bacterial culture was inoculated into 100 µl of brain heart infusion (BHI) broth, serially diluted to obtain the selected optical density having bacterial concentration of 10^6 CFUs/ml. 150µl of the bacteria suspension was pipetted onto the disc surface so that it stays onto the surface and does not leak beyond the edges of the disc. Before incubating the discs anaerobically, half of the control discs were washed with 850µl of sterile phosphate buffered saline (PBS). To ensure that PBS completely washes the discs, the same PBS was collected and pipetted four times. Finally, the collected PBS was serially diluted in BHI, plated in duplicates onto FAA plates and incubated anaerobically. The number of colonies was counted after 48 h and repeated again after 4 days. The plates with the other discs (other half of the control, 5% and 10% EgMA composites) were incubated anaerobically at 37 °C for 24 h. After incubation they were washed with sterile PBS, the collected PBS was serially diluted, plated in duplicates onto FAA plates and incubated anaerobically for quantitative viable counts as mentioned above.

The number of viable bacteria recovered was determined according to the following equation:

$$N = \frac{C \,.D}{A} \qquad (9)$$

where, N is the number of viable bacteria recovered per cm^2 per test specimen; C is the average plate count for the duplicate plates; D is the dilution factor the plates counted; A is the surface area of test specimen in cm^2.

The antibacterial activity R was calculated using the following equation:

$$R = (U_t - U_0) - (A_t - U_0) = Ut - At \qquad (10)$$

where, R is the antibacterial activity; U_0 is the average of the logarithm of the number of viable bacteria, in cells·cm^{-2}, recovered from the control specimens immediately after

inoculation; *Ut* is the average of the logarithm of the number of viable bacteria, in cells cm^{-2}, recovered from the control test specimens after 24 h; *At* is the average of the logarithm of the number of viable bacteria, in cells cm^{-2}, recovered from the EgMA containing test specimens after 24 h.

4.2.2.5 Statistical analysis

The mean values of water sorption, solubility, D, θ, Y_s and logarithmic CFU count/cm^2 were calculated and differences was analysed by one-way ANOVA, followed by Tukey's post-hoc analysis at significance level of $P<0.05$. Independent-samples *t*-test was used to compare the antibacterial activity (*R*) of composites between two different concentrations of EgMA. Standard deviation (SD) of each experiment were calculated and represented in brackets alongside the respective mean value.

4.2.3 Results

4.2.3.1 Water sorption and solubility

Plots of $Mt/M\infty$ versus $t^{1/2}$ for the composites tested during water sorption in the first and second cycle are shown in Fig 4.2-2. All curves were fit to a linear regression during the early stages of diffusion-controlled uptake of water; which allows the calculation of the diffusion coefficients from the initial curve slope.

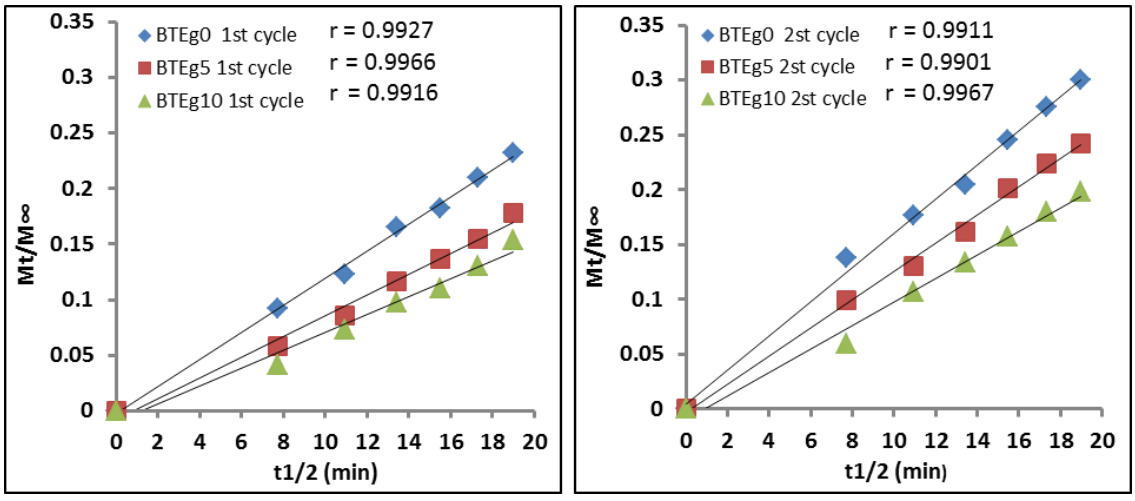

Figure 4.2-2 Early water uptake behaviour and linear fit of the composites during the first and second sorption cycle

The water uptake weight percentage, water sorption, solubility values and the sorption diffusion coefficient of the experimental composites during the first and the second cycle are summarised in Fig. 4.2-3 and Table 4.2-2. In both cycles, all composites reached equilibrium within the first week of immersion; the water sorption and diffusion coefficients values were significantly reduced with increasing content of EgMA (P<0.05). The values of the diffusion coefficients calculated were larger during second sorption cycle. For water solubility, although slight reduction was observed for EgMA containing composites, the statistical analysis showed significant reduction in the solubility value of BTEg10 composite only during the second cycle when compared with the control (BTEg0) composite.

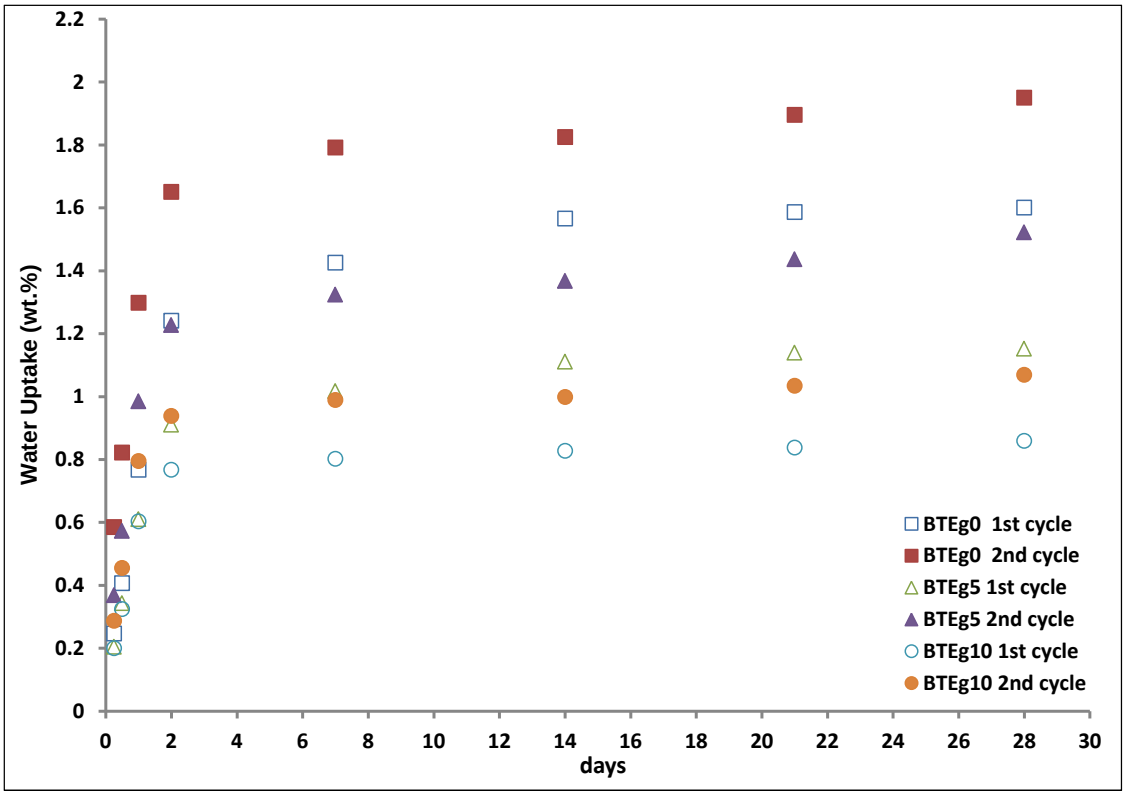

Figure 4.2-3 Mass change in percentage of the composites during immersion in water over 28 days (first and second sorption cycle)

Table 4.2-2 Mean (SD) values of water sorption, solubility and sorption diffusion coefficient for composite materials, for a total immersion period of 28 days

Composites	Water sorption ($\mu g/mm^3$)	Water solubility ($\mu g/mm^3$)	Diffusion coefficient (10^{-8} cm^2 s^{-1})
BTEg0			
1st cycle	**34.8 (0.5)**	**4.6 (0.8)**	**2.87 (0.02)**
2nd cycle	**39.0 (0.8)**	**2.3 (0.4)**	**4.08 (0.03)**
BTEg5			
1st cycle	**27.1 (0.8)***	**4.5 (0.3)**	**2.21 (0.03)***
2nd cycle	**29.7 (0.7)****	**1.5 (0.7)**	**3.26 (0.01)****
BTEg10			
1st cycle	**20.4 (0.8)***	**4.2 (0.3)**	**1.62 (0.01)***
2nd cycle	**21.1 (0.2)****	**1.1 (0.5)****	**2.89 (0.02)****

Differences were statistically significant with respect to control BTEg0 composite (* 1st cycle, ** 2nd cycle) (*P*<0.05)

4.2.3.2. Wettability of the composites

The contact angle (θ) values and surface free energy (Y_S) are summarised in Table 4.2-3. The addition of EgMA in the composite formulation significantly increased the contact angle measurements with both tested liquids which resulted in a significant reduction of the calculated surface free energy (P<0.05), indicating the higher hydrophobicity of composites surfaces.

Table 4.2-3 Contact angle and solid surface free energy components for the experimental composites

Composites	Θ (H_2O)	Θ (CH_2I_2)	Y_S (mN/m)	Y_S^d (mN/m)	Y_S^P (mN/m)
BTEg0	**54.9(2.9)**	**43.2(1.6)**	**54.3 (1.9)**	**38.6**	**15.6**
BTEg5	**64.7(3.5)***	**46.8(3.3)***	**47.7 (1.2)***	**36.7**	**11**
BTEg10	**68.8(3.4)***	**49 (2.5)***	**44.8 (1.7)***	**35.5**	**9.3**

θ (H_2O) = water contact angle; θ (CH_2I_2) = methylene iodide contact angle; and *Y*s = surface free energies with their dispersive (Y_S^d) and polar (Y_S^P) components.
*** Differences were statistically significant with respect to control BTEg0 composite (*P*<0.05).**

4.2.3.3. Antibacterial assay

4.2.3.3.1. Agar Diffusion Test

The results of this test showed that the lack of inhibition zone detected around the specimens of the 3 test composites against the 3 bacteria tested (*E. faecalis*, *S. mutans* and *P. acnes*), indicating that there was no elution of any antibacterial component from the bulk specimens.

4.2.3.3.2. Antibacterial activity of the composites surface

Fig. 4.2-4 illustrates the respective plate images of *E. faecalis*, *S. mutans* and *P. acnes* strains at dilution factor (-2) and after 24 h incubation on composite surfaces according to the ISO 22196:2007.

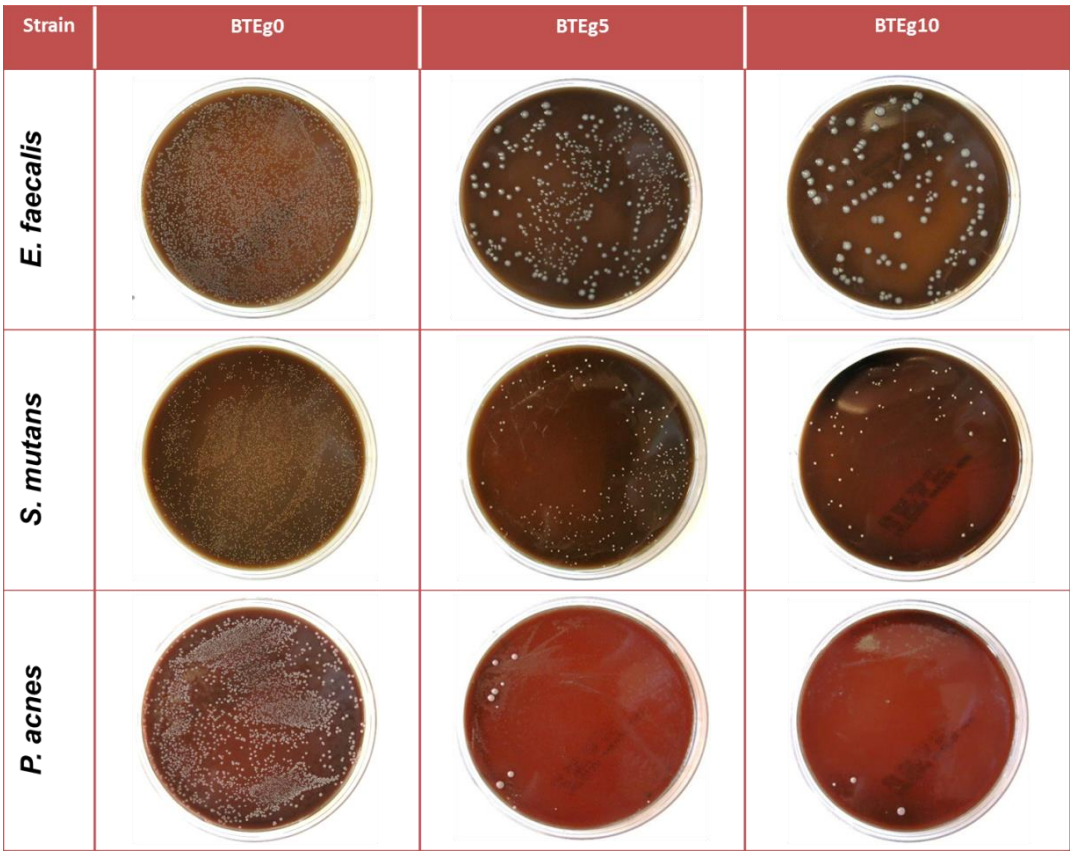

Figure 4.2-4 Respective plate images of composites surface antibacterial activity assay according to the ISO 22196:2007 after 24 h incubation (dilution factor -2)

The number of bacteria as $\log_{10}$ CFU per test composite and the calculated *R* values representing the antibacterial activity of composites are shown in Fig. 4.2-5 and Table 4.2-4 respectively. From the results, it can be seen that for control group the $\log_{10}$ CFU recovered were significantly higher than those recovered immediately after inoculation ($P<0.01$). The number of colonies of all tested bacteria was reduced by the addition of EgMA into the formulation of the composites and the $\log_{10}$ CFU of these composites were significantly lower as compared with the control composites ($P<0.01$).

No significant difference was observed in antibacterial activity between BTEg5 and BTEg10 composites (P>0.05) with R values of the antibacterial activity R ranged between 2.7 and 6.1 following the order *E. faecalis* < *S. mutans* < *P. acnes*.

Table 4.2- 4 Antibacterial activity (R) of experimental composites tested according to the ISO 22196:2007 after 24 h incubation

	E. faecalis		*S. mutans*		*P. acnes*	
	N (CFU/cm^2)	***R*** = U_t - A_t ($\log_{10}$ CFU)	***N*** (CFU/cm^2)	***R*** = U_t - A_t ($\log_{10}$ CFU)	***N*** (CFU/cm^2)	***R*** = U_t - A_t ($\log_{10}$ CFU)
BTEg0	1.3×10^8	$U_t = 8.1$	6.8×10^7	$U_t = 7.8$	5.8×10^7	$U_t = 7.7$
BTEg5	3.7×10^5 *	2.7	2.3×10^3 *	4.6	1.1×10^2 *	5.7
BTEg10	2.2×10^5 *	2.8	1.0×10^3 *	4.9	4.7×10^1 *	6.1

***N*: the mean number of viable bacteria recovered per cm^2; *Ut*: is the average of the logarithm of the number of viable bacteria, in cells·cm^{-2}, recovered from the control test specimens after 24 h; *At*: is the average of the logarithm of the number of viable bacteria, in cells·cm^{-2}, recovered from the EgMA containing test specimens after 24 h. * Significant differences with respect to control BTEg0 composite (P<0.01).**

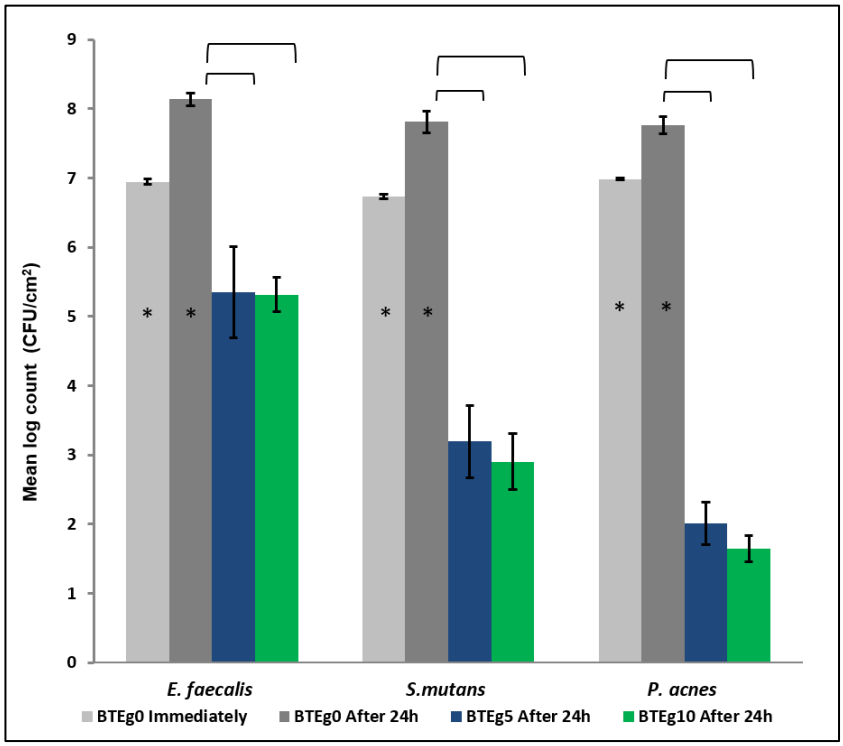

Figure 4.2-5 The mean $\log_{10}$ CFU/cm^2 of tested bacterial species on the experimental composites. * indicates significant differences between control groups (BTEg0) recovered immediate and after 24 h incubation (P<0.01). The connection with horizontal lines indicates significant differences between groups after 24 h incubation (P<0.01)

4.2.4 Discussion

In present study, the new composite formulations bearing the eugenyl derivative monomer showed an effective bacteriostatic activity against different oral microorganisms. The novelty of these formulations originates from the immobilisation of eugenol molecule within the resin matrix, preventing its potential side effect against surrounding tissue and enhancing the hydrolytic stability, while maintaining the biological properties of eugenol.

The inhibitory effect of eugenol against a variety of oral bacteria has been reported (Kaplan *et al.* 1999), however, one of major limitations of eugenol applications in dentistry is derived from the antioxidant character of the unreacted molecules of eugenol which inhibit the free radical polymerisation of dental composite resin materials and can also produce tissue irritation. By modifying the chemical structure of eugenol, EgMA monomer was synthesised after acylation reaction of eugenol with methacrylic chloride, the new derivative has the ability to copolymerise with other methacrylate monomers and immobilise the antibacterial eugenol moieties in the polymer backbone without the inhibitory effect characteristic of the phenol derivatives (Rojo *et al.* 2009). Moreover, cytocompatibility of EgMA containing polymer matrices has been reported earlier with the absence of any toxic eluants (Rojo *et al.* 2006).

The use of EgMA monomer in the formulation of the experimental composites offers several advantages in terms of complete miscibility with Bis-GMA/TEGDMA mixtures, improved mechanical properties and better processability and handleability of the corresponding composite pastes (Almaroof *et al.* 2016a). In addition, the immobilisation of the antimicrobial agents offers an additional benefit in comparison with other systems where the mode of action involve leachable antimicrobial agents such as chlorhexidine, which lacks the miscibility with other dental monomers and thus leading to adverse influence on mechanical properties, increased water sorption, porous structure and short term effectiveness (Jedrychowski *et al.* 1983).

Water sorption and solubility of polymeric composites are of importance for dental applications. The physical and mechanical properties of resin composite materials may be significantly altered by the effects of water sorption and component elution. Fluid uptake in an oral environment also leads to harbouring of bacteria within these composites that eventually lead to discolouration and failure. Several factors, including monomers hydrophilicity, cross-link density and the presence of fillers can affect the water sorption of resin based composite materials (Ferracane 2006).

The water uptake of the experimental formulations with and without EgMA showed values that were in agreement with previous data on commercial composite core formulations (Martin *et al.* 2003; Zankuli *et al.* 2014) and fulfilled the requirements for dental applications in accordance to ISO 4090 standard requirements that limit the values of water sorption and solubility to a maximum of 40 µg/mm^3 and 7.5 µg/mm^3 respectively. The reduction in water sorption and diffusion coefficients values with increasing content of EgMA monomer was due to monomer hydrophobicity and ability to form slightly cross linked structures (Rojo *et al.* 2008b) which reduced the water permeability of the polymer by decreasing the free space and thereby the swelling of the polymer. This finding was consistent with other studies in which EgMA was copolymerised with other methacrylate monomers, ethyl methacrylate (EMA) (Rojo *et al.* 2008b) and 2-hydroxyethyl methacrylate (HEMA) (Rojo *et al.* 2008a). It has been reported also that the incorporation of HA fillers reduced the water uptake of methacrylate based dental composites especially when the HA particles were surface treated with a silane coupling agent (Deb *et al.* 1995). Therefore, the use of HA particles within our experimental formulations had a distinct contribution in lowering water uptake, limiting the extraction of unreacted components which cause weight loss and adversely effects mechanical properties and longevity of these materials (Musanje *et al.* 2001).

The water uptake indicates that the sorption behaviour of the composites follow Fickian diffusion and the coefficients of diffusion obtained in this study were comparable to published values obtained from resin composites formulated with silanised HA filler

(Santos *et al.* 2002) with higher values of the diffusion coefficients for second sorption cycles (Table 4.2-2). This trend was also observed for conventional resin composites in previous studies (Santos *et al.* 2002; Sideridou *et al.* 2004) and can be explained by the fact that the movement of the water molecules in the first sorption is hindered from the eluting monomer molecules. The water sorption values were also higher in the second sorption cycle due to the loss of unreacted components and the solubility of the experimental composites correlated with their water sorption behaviour as expected.

The contact angle and surface free energy are important parameters for determining the hydrophobicity of the materials and their interactions with medium and usually related with its water sorption. There is an effect of SFE substrates on bacterial adhesion, which has been critically discussed in the literature, however there is no clear consensus. Bacterial adhesion is a complex phenomenon and is related to the surface energy of a solid. However, this relationship is not a linear correlation since the chemical composition of the surface tends to govern the interaction which depends on both the chemistry of the solid and immersion liquid and additionally the type of bacteria and growing media. This has resulted in conflicting reports with reports suggesting that materials with low SFE result in less bacterial adherence (Ferracane 2006; Buergers *et al.* 2009); whilst other contrary reports found that bacterial adhesion decreased with increasing surface energy of substrates (Absolom *et al.* 1983; Mceldowney and Fletcher 1986; Mei *et al.* 2009). The addition of EgMA monomer containing a substituted aromatic ring into the formulation significantly increased the hydrophobicity leading to an increase of the surface contact angle and reducing the SFE values from 54.3 mN/ m for the control, to 47.7 and 44.8 mN/ m for composites containing 5% and 10% EgMA respectively. The antibacterial activity of the experimental composites is derived from the pendent eugenyl residue from polymer network (Fig. 4.2-1). Therefore, the ability of these composites to reduce or inhibit bacterial growth is highly dependent on the direct contact between eugenyl residues of the composite surface and the bacteria.

According to the bacterial adhesion theory DLVO (Derjaguin 1993), the total interaction energy between the bacteria and the solid surfaces is the sum of several interaction components such as Van der Waals attractive interaction, electrostatic double-layer repulsive component, Lewis acid–base component. These interactions are entirely related to the SFE of substrates. It was noted that the greater hydrophobic character of EgMA containing composites, presented a lower SFE, improved the total interaction energy with the bacteria and resulted in a higher accessibility to the eugenyl moieties responsible for the bacteriostatic activity. This could explain the reduction in number of CFU of bacteria tested and is in agreement with other studies which found that the total interaction energy between the bacteria and the substrate is linearly increased with the decrease of the surface energy (Absolom *et al.* 1983; Liu and Zhao 2005).

Agar diffusion test findings were consistent with other studies on bactericide-immobilised materials, such as zinc oxide nanoparticles (Hojati *et al.* 2013) and methacryloyloxydodecyl pyrimidinium bromide (MDPB) (Imazato *et al.* 1994), incorporated into resin composite with no leachable antibacterial agents exhibiting surface inhibition properties. The lack of inhibition zones around composites discs containing EgMA confirmed their non-releasing behaviour as the inhibition zones can only be formed by the diffusion of antimicrobial material indicating that the antibacterial activity of the composites is not through the release of agent to the medium but is associated with surface contact.

The surface antibacterial activity test is a standardised quantitative assessment of the inhibitory effect on bacteria that contact the composite surface on which the EgMA is immobilised. It is clearly evident from the results that composites with eugenyl moieties had a highly significant inhibitory effect on the three types of bacteria tested, indicating that the chemically bound monomer has the capability to reduce or inhibit the colonisation of these bacteria which come into contact with composites surfaces. The significant differences between the numbers of CFU of the control samples recovered immediately and after 24 h incubation indicating the favourable incubation growth

condition for the bacteria and confirming the validity of the test. Composite containing 10% EgMA showed the highest inhibitory effect and reduced the number of *S. mutans* and *P. acnes* colony more efficiently than 5% EgMA composite. However, no significant difference was found between the two concentrations for all tested bacteria. As mentioned above the effectiveness of the composites depend mainly on the contact between the eugenyl residue of the monomer pendent from polymer network and the bacteria. Therefore, the amount of these residues distributed on the surface of the sample may not differ greatly by increasing the monomer content from 5 to 10 wt.%.

The exact mechanism of the bacteriostatic properties of immobilised eugenol containing materials remains unclear. However, eugenol below its minimum inhibitory concentration (sub-MIC) has been demonstrated to reduce the virulence properties such as adherence and biofilm formation of the cariogenic bacteria (Li *et al.* 2013; Adil *et al.* 2014). In addition other studies have demonstrated the effect of eugenol on a variety of oral bacteria causing disruption in cell membrane permeability (Ultee *et al.* 2002) and proton pumps (Sikkema *et al.* 1994) that reduce microbial resistance and biofilm formation on dental materials, which explains the well-known bacteriostatic properties shown in other eugenol containing materials commonly used in dentistry such as zinc oxide eugenol cements and eugenol based root canal sealers (Mickel *et al.* 2003).

The value of the antibacterial activity *R* of EgMA composites in this study was higher than 2.0, the minimum accepted value for materials to exhibit an effective antibacterial surface (Bazant *et al.* 2014). The difference in *R* values against the three tested types of bacteria can be attributed to respective virulence of each species and to the differences in the chemical composition and structure of the bacteria cell walls that resulted in different bacterial sensitivities toward EgMA.

Lower *R* values achieved against *E. faecalis*, a facultative anaerobe, which is one of the resistant bacteria commonly found in the root canals of teeth with endodontic treatment failure (Stuart *et al.* 2006). *E. faecalis* has displayed resistance to a wide range of

antibiotics (Kayaoglu and Ørstavik 2004). Moreover, it has been found that the effective proton pump mechanism which maintains optimal cytoplasmic pH levels of these species may participate in their resistance to the antimicrobial effects of calcium hydroxide (Evans *et al.* 2002).

S. mutans virulence factors include its ability to synthesise adhesive glucans (Xiao and Koo 2010) and generate acids that result in the demineralisation of dental tissues, thereby initiating dental caries. Studies in literature reported that eugenol can effectively suppress the virulence of *S. mutans in vitro* by reducing the total mass of microorganisms and by virtue of its anti-adherence against this bacterium (Xu *et al.* 2013). Eugenol at sub-MIC concentrations has the ability to inhibit the formation of adhesive glucans synthesised by glucosyltransferases (GTFs) that provide specific binding sites for bacterial colonisation on the tooth surface and binding to each other (Xiao and Koo 2010).

The testing of the antibacterial activity of the composite showed an excellent performance against *P. acnes* reported the highest *R* values. *P. acnes*, an opportunistic pathogen in refractory endodontic infection, may be associated with contamination of dental materials during the root canal filling procedure and has been isolated from infected root canals (Niazi *et al.* 2010). This species has been found to have high sensitivity to essential oil components that may adhere to the bacterial surface at low concentration (Fu *et al.* 2007).

4.2.4 Conclusions

The incorporation of EgMA monomer as immobilised bactericidal moieties within polymerisable formulations provides a novel approach to develop resin composite materials with intrinsically antibacterial activity against oral bacteria commonly associated with coronal and endodontic restorations failures and therefore indicating their potential for use in different clinical applications.

4.3 Supplementary information on bonding ability and cytotoxicity of EgMA containing composites

4.3.1 Introduction

A major drawback of methacrylate-based dental composites is the polymerisation shrinkage that results in the development of contraction stresses between the composite and the tooth structure, especially inside the canal because of the high configuration factor (Tay *et al.* 2005). These stresses severely strain the bonding interfaces, leading to marginal gaps and subsequent bacterial invasion and recurrent caries. Therefore, the bond strength of the luting materials to root canal dentine is an important factor for the restoration of endodontically treated teeth bonding. Failure of adhesively luted fibre posts occurs more often through decementation (Naumann *et al.* 2012).

On the other hand, the cytotoxicity evaluation is also one of the critical tests for any new materials such as composites which are intended to be used in human, especially antibacterial composites designed for biomedical applications, which should be minimally cytotoxic to host tissues. For post placement restoration of ETT, polymeric resin composites, cements and adhesive materials are usually applied inside the root canal space that interfaces with adjacent tissues. Since curing these materials is usually not complete, leachable substances such as unreacted monomers will penetrate through the dentinal tubules or release into the gingival tissue and saliva, resulting in an adverse local reaction or even systemic effects (Schmalz 1998).

Therefore, this section involves further characterisation of the experimental resin composites by testing, *in vitro,* the influence of monomer incorporation on bonding ability and biocompatibility of these composites.

4.3.2 Supplementary methods

4.3.2.1 Bonding ability to root canal dentine

The bonding performance of the experimental (BTEg0, BTEg5 and BTEg10) and the commercial composites to root canal dentine was evaluated through push-out bond strength test and confocal laser scanning microscopy (CLSM) observation.

Thirty-six human single-rooted premolars extracted after obtaining informed consent from the patients and following a protocol approved by an institutional review board were used in this study (Research Ethics Committee Reference Number 14/LO/0123). All teeth were stored at 4 °C in distilled water and used within one month. Teeth were randomly and equally assigned to four groups based on the resin composite materials used in this study (three experimental and one commercial, n=9), with three specimens from each group reserved for confocal microscopy analysis. The crown was sectioned at the cemento-enamel junction using a low-speed, water-cooled diamond saw microtome (Isomet 1000, Buehler, Lake Bluff, IL, USA). The teeth were endodontically treated and their roots were mounted vertically in acrylic resin block using an aligning device. After 24 h storage at 37 °C in relative humidity, the first 8 mm of the canal was shaped with a cylindrical flat end diamond bur (Komet 837/016, Lemgo, Germany) so that a standardised cavity of 2 mm in diameter was prepared in the coronal and middle portion of the root canal. In all groups, a self-etch adhesive (CLEARFIL Universal Bond™, Kuraray, Japan) was used in accordance with the manufacturer's instructions. The bonding agent was applied to the entire root canal with the applicator brush and rubbed for 10 s, then dried with a gentle air blow for 10 seconds and light-cured for 40 s using a dental light-curing unit at 600 mW cm^{-2} (Optilux, Demetron Res Crop, Danbury, USA). The bonded cavities were then directly filled with a resin composite. For the experimental composite groups, equal amounts of each paste were mixed on a glass slab and the mixture was then loaded into a 1 ml syringe with a guide tip. The composite was injected into the post-space and light-cured for 40 s. The commercial composite (Clearfil™DC Core plus, Kuraray, Japan) was applied according to the manufacturer's

instructions. The root segment was then placed in individually labelled containers in relative humidity at 37 °C. After 24 h, the bonded roots were transversely sectioned to create 1.5 mm thick root slices. The thickness was verified using a digital electronic caliper, with the top root slices discarded to avoid the influence of excess material, producing four root slices from each root (two coronal and two middle) for subsequent push-out bond strength tests. The push-out force was applied in an apical-coronal direction using a cylindrical plunger with a diameter of 1.8 mm attached to a universal testing machine (Instron model 5569A-Series Dual Column, High Wycombe, UK) at a crosshead speed of 0.5 mm/min until failure (Fig. 4.3-1).

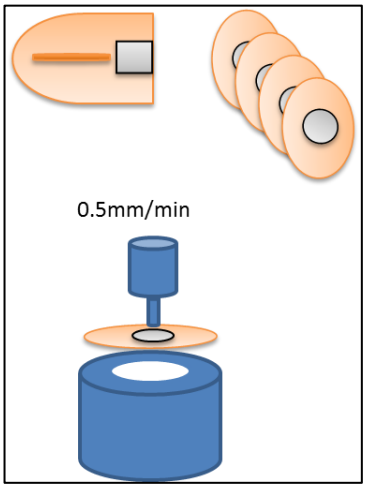

Figure 4.3-1 Specimen preparation for the push-out test

The maximum load at failure was recorded in Newton (N) and was converted to MPa by dividing the applied load by the bonded area, using the following equation:

Push-out bond strength = force/ π x diameter x thickness

The data were analysed by two-way ANOVA (SPSS version 20).

For CLSM-Interface evaluation, 0.1 wt% fluorescein dye (Sigma-Aldrich, UK) was added to the adhesive, while 0.1 wt.% Rhodamine B (Rh B: Sigma- Aldrich, UK) was added into the composites core materials. A further three specimens from each group were bonded and filled, as previously described, with these labelled materials, and employed for the confocal microscopy analysis as reported by Almaroof *et al.* (2017) (section 5.1.2-9).

4.3.2.2 Biocompatibility study

Cytotoxicity assays are used as initial screening tests to evaluate the biocompatibility of materials; several methods have been applied for this purpose. In this study, the cytotoxicity of the resin composites was determined quantitatively by MTT cytotoxicity assay. This assay has been used extensively to evaluate the cell viability and determine cytotoxicity of dental materials because of its simplicity, accuracy and reliability. Additionally, a live/dead cell viability assay was also used for qualitative investigation.

Sample preparation and sterilisation: Specimens of the experimental composites were prepared using a Teflon mould to produce discs of 10 mm diameter and 1 mm thickness as described in section 4.2.2.2. The specimens were allowed to set for 24 h in a humid atmosphere and aseptic conditions. Samples were then wiped with 70% ethanol in water and sterilised by ultraviolet light for 40 min from both sides using the UV lamp of a class II biological safety cabinet (Nuaire™, Plymouth, USA).

4.3.2.2.1 MTT (methyl tetrazolium) assay

MTT assay was used to evaluate cell viability at 24 h and 48 h according to the International Standard ISO 10993-5. This assay is based on the ability of the mitochondrial enzyme succinate-dehydrogenase within the viable cells to convert the yellow water-soluble tetrazolium salt MTT into a purple coloured formazan compound by addition of a detergent such as dimethyl sulfoxide (DMSO). The absorbance of formazan produced is directly proportional to the amount of living cells present, whose colour can be quantified by spectrophotometric means (Mosmann 1983).

Composite eluents were obtained by immersing the samples in 3 ml of sterile fibroblast medium within bijou vials, which were then placed onto a roller at room temperature. The supernatants were collected at 24 and 72 h time points and refrigerated at -20°C to be used for MTT cytotoxicity measurements as described in section 3.2.5.5.

4.3.2.2.2 Live/dead cell assay

HGF cells viability on composites was analysed using a Live/Dead kit (L-3224) from Invitrogen at time points 1 and 3 days. Samples (two from each group) were removed and placed in new 24-well plates; cells were seeded at a density of $1x10^5$ cells/ml per well onto the samples and incubated at 37 °C, 5% CO_2. At each time point, media was removed and replaced with 200 mL stock solution of 1 mM calcein AM and 2 mM ethidium homodimer in PBS, which was prepared according to the live/dead assay kit instructions. Calcein AM hydrolysis in live cells produces a green fluorescent signal, while ethidium homodimer is excluded from live cells and produces a red fluorescent signal only in compromised (dead) cells. The materials were observed and imaged using a fluorescence microscope (Olympus IX51) at 20x magnification. The live green signal was viewed/imaged using a 480 nm band pass excitation filter with a 510 nm bandpass emission filter. The dead (red) signal was viewed with a 520 nm bandpass with a 560 nm bandpass filter for emission.

4.3.3 Results and discussion

4.3.3.1 Bonding ability

Data from the push-out tests are presented in Table 4.3-1. Two-way ANOVA analysis revealed that there were no statistically significant differences among the composites (P=0.094) indicating that the addition of EgMA had no adverse effect on push-out bond strength. However, this effect referred exclusively to the difference among the overall means of the coronal and middle regions. For all composites, significant differences in bond strength between coronal and middle regions were found with no interaction between the composite and region (P=0.73).

Table 4.3-1 Comparison of the mean push-out bond strength (BS) values and the analysis of failure modes (n=24)

Composite	Bond strength values (mean ± SD)			Failure mode (%)		
	Regional BS		Total BS	Adhesive	Mixed	Cohesive
	Coronal	Middle				
BTEG0	12.9 ± 3.5	5.0 ± 1.2	9.0 ± 4.7	62	34	4
BTEG5	13.0 ± 3.4	7.2 ± 2.6	10.17 ± 4.2	58	34	8
BTEG10	13.8 ± 3.8	7.2 ± 2.34	10.5 ± 4.6	54	38	8
Clearfil™DC	11.3 ± 2.3	6.2 ± 2.02	8.82 ± 3.3	62	30	8

*** No statistical differences in total bond strength among the composites (*P*<0.05)**

In this study, the post cavities were filled with the resin composite materials without posts placement to investigate solely their bond strength to root dentine excluding the composite-post interface. The push-out bond strength values obtained from the experimental composites were also comparable to those of the commercial material and within the range of previous studies that evaluated the properties of various dual-cure resin composite core materials (Lee *et al.* 2014; Zavattini *et al.* 2014). The microscopic evaluation of the failure mode revealed that the majority of failure patterns occurred as interfacial failure between dentine surface and resin composites, which is in accordance with those of previous reports (Erdemir *et al.* 2010; Schetini *et al.* 2014). More complicated failure patterns such as mixed failure and cohesive failure within the resin were found when bond strengths were higher.

Additional detailed information regarding the distribution of resin composites and adhesive interface were provided by the CLSM method. Fig. 4.3-2 is a representative selection of the CLSM observations. The interface between the self-etch adhesive and root dentine showed a clear hybrid layer located underneath a thick adhesive layer. In coronal and middle root dentine, the presence of resin tags was evident in both control and EgMA containing composites.

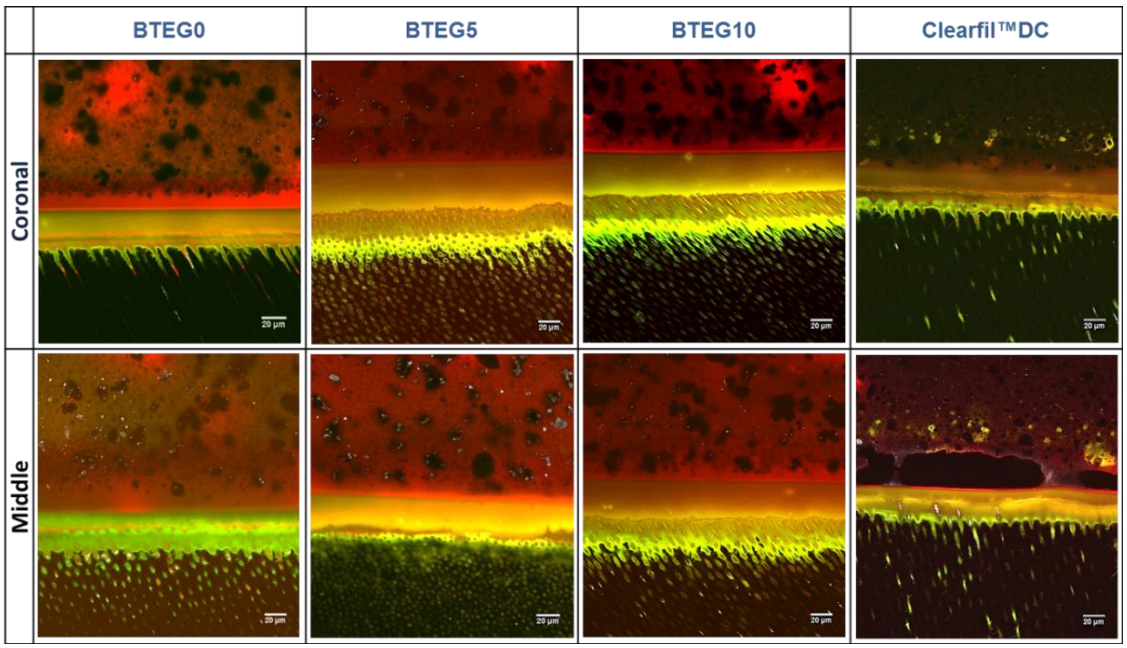

Figure 4.3-2 Representative confocal laser scanning microscopy (CLSM) images demonstrating all tested resin composites, adhesive (Clearfil Universal Bond) and dentine taken from the coronal and middle regions of the root. The adhesive, which was labelled with fluorescein dye, showed green fluorescence, while the resin composites that were stained with rhodamine B exhibited red fluorescence. The resin diffusion into adhesive layer and its mixture with labelled adhesive produced orange colour fluorescence.

Although the bond strength results of the experimental and the commercial composites were comparable, the CLSM assessment in all the samples examined showed an optimal integration between the adhesive layer and the experimental composites with no air bubbles or voids in the composites layer. On the other hand, in two samples from the commercial material, several micro-bubbles could be observed occupying the composite layer, especially in the middle region of the root. This could be attributed to the difference in viscosity between the experimental and the commercial composites. The lower viscosity can facilitate the application and the distribution of the luting material and reduce air bubble formation (Aksornmuang *et al.* 2014).

4.3.3.2 Biocompatibility

The results obtained from the MTT assay are shown in Figs. 4.3-3 and 4. In all groups the cell viability was never below 70% of the control, the minimum cell viability percentage below which the material has a cytotoxic potential (ISO 10993-5). The cell viability after 24 h exposure to the 72 h extracts from the test materials showed significant changes in comparison to the control. However, at 48 h cell exposure, it speculated that

the initial reaction is stopped and a comparable percentage of cell viability was observed for all experimental composites. No interaction between time and groups was found using a linear regression test (*P*=0.1).

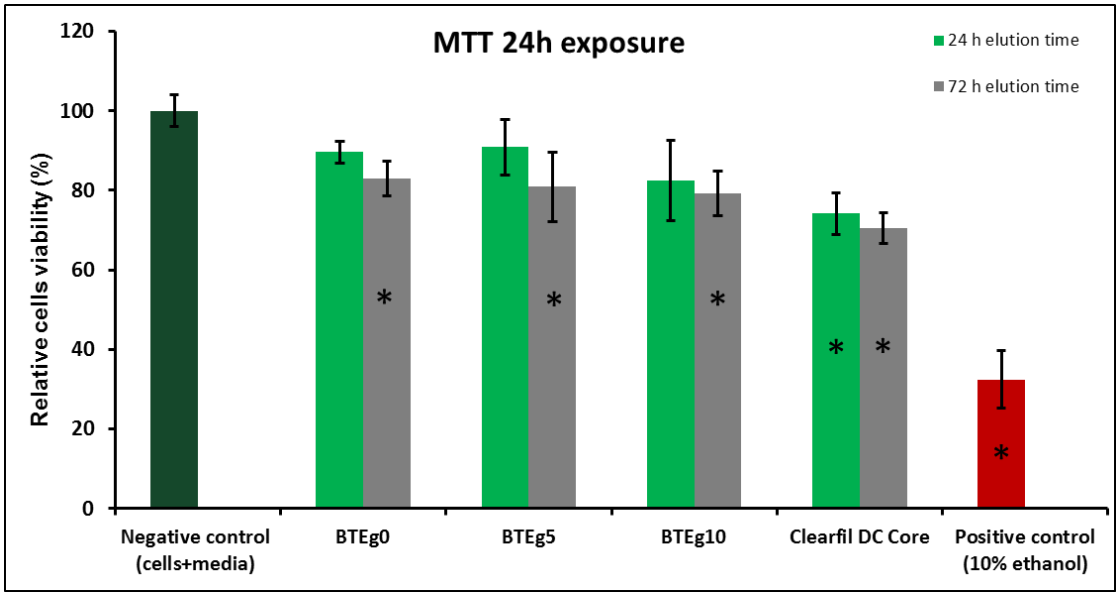

Figure 4.3-3 Viability of human gingival fibroblast (HGF) cells following exposure for 24 h, detected at 24 h and 72 h eluted media from experimental and commercial composite materials. The relative cell viability is presented as percentage of negative control cells. *Denotes significant difference (*P*<0.05) when compared with control

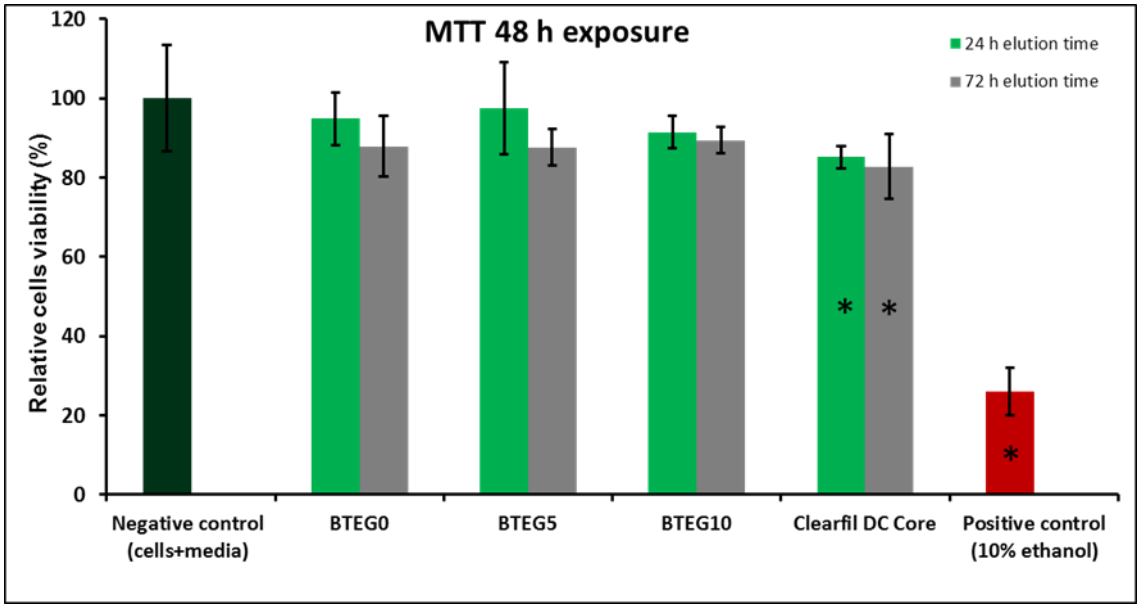

Figure 4.3-4 Viability of human gingival fibroblast (HGF) cells following exposure for 48 h, detected at 24 h and 72 h eluted media from experimental and commercial composite materials. The relative cell viability is presented as percentage of negative control cells. *Denotes significant difference (*P*<0.05) when compared with control

For both exposure times, no significant cytotoxicity was found between 24 h and 72 h elusion time. This finding was expected since leaching is essentially complete in 24 h and in agreement with previous studies (Ferracane and Condon 1990; Moharamzadeh *et al.* 2007). However, the experimental composite was found to be more biocompatible ($P<0.05$) than the commercial material and exhibited no deleterious effects on the metabolic activity of cells at 48 h exposure time points compared to the negative non-toxic control group. Furthermore, the comparison among the experimental groups showed that the cytotoxicity of BTEg5 and BTEg10 composites was statistically non-significant compared to the BTEg0 control at both 24 h and 72 h elution time points. These findings indicate that the monomer concentration had no significant influence on HGF cell viability.

MTT results are supported by the images obtained from the live/dead assay test shown in Fig. 4.3-5. It is clear that cell viability on the surface of EgMA containing composites was equivalent to that on the control BTEg0 surface at one and three-day exposure points. The figure also qualitatively shows a good colonisation of the cells which appeared well spread and flattened over all materials, indicative of a good cytocompatibility.

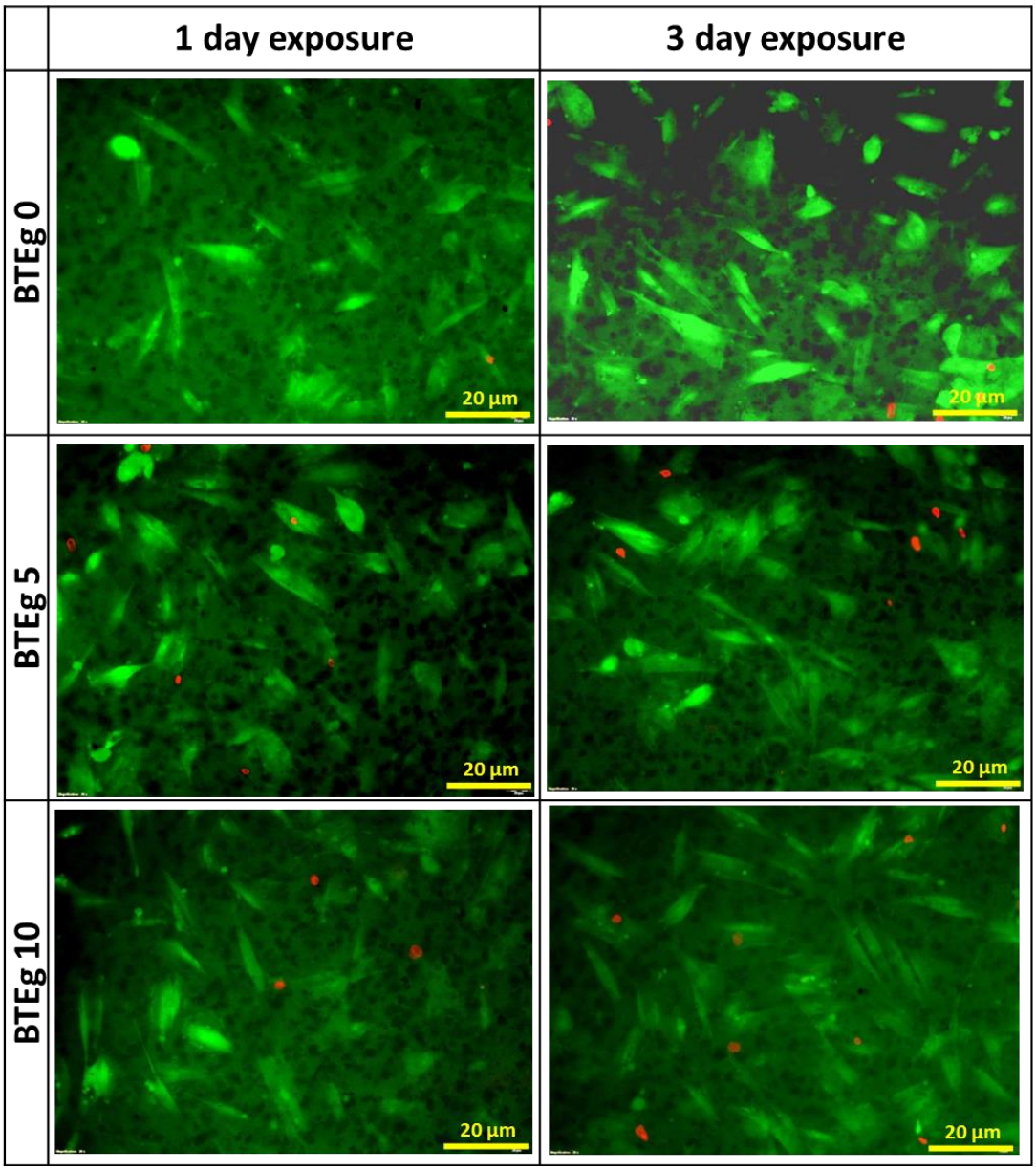

Figure 4.3-5 Live/dead cytotoxicity assay of human gingival fibroblast at a density of $1x10^5$ cells/ml, one day and three days after introduction onto the experimental composites

It is known that eugenol at low concentrations can exert anti-inflammatory and local anaesthetic effects on the dental pulp; however, high eugenol concentrations are cytotoxic (Markowitz *et al.* 1992). Free eugenol may release from ZOE temporary filling material and diffuse through dentine or into the saliva. Eugenol and related compounds have been shown to have a high affinity for plasma membranes because of their lipid solubility, which could contribute to cell damage (Manabe *et al.* 1987).

Earlier, the IC_{50} (mmol/L) of EgMA monomer and eugenol was determined and their values were 3.70 and 2.60, respectively (Rojo *et al.* 2006). However, it is important to mention that EgMA was added in small amounts (5 and 10 wt.%) to high molecular weight polymers (Bis-GMA and TEGDMA) and polymerised in a cross-linked network because of the presence of polymerisable C=C methacrylate bonds in its structure; therefore, any unreacted monomer that may arise would be predominantly due to these major components.

Residual monomers and other components are released from polymerised resin-based dental materials and may induce adverse local reaction or even systemic effects (Schmalz 1998; Kaga *et al.* 2001). Monomers used in dental restorative materials have been found to exhibit a variety of toxic effects on gingival fibroblasts, and the rank of five monomers tested are Bis–GMA > TEGDMA >DMAEMA > HPMA > HEMA for MTT assay (Issa *et al.* 2004). Furthermore, the cytotoxicity of EgMA monomer has also been studied earlier (Rojo *et al.* 2006); its effect was similar to EMA monomer commonly used in the formulation of restorative dental materials. Therefore, immobilisation with a non-leachable antimicrobial agent, which endow the composites with antibacterial activity, may also be advantageous in preventing its potential toxic effect on surrounding tissue.

The different cytotoxicity results between the experimental and the commercial composites may be explained by the differences in their chemical composition. The presence of hydroxyapatite filler enhanced the cytocompatibility of the experimental composites. This finding is supported by previous studies, wherein the biocompatibility of HA-reinforced composites has been reported (Di Silvio *et al.* 2002; Zhao *et al.* 2004). It is also interesting to note that the inclusion of ZrO_2 into the composite produced no significant changes on the metabolic activity and growth of cells.

Chapter 5 Incorporation of eugenyl methacrylate (EgMA) antibacterial monomer into dental adhesives systems for application in endodontic restorations

This chapter includes a full characterisation of two commercial dental adhesive systems (self-etch and total etch) modified with EgMA antibacterial monomer for their application in bonding of endodontic restorations to root canal dentine (section 5.1, Almaroof *et al.* 2017). Further supplementary information on the durability of EgMA modified adhesives is presented in section 5.2.

5.1 Evaluation of dental adhesive systems incorporating an antibacterial monomer eugenyl methacrylate (EgMA) for endodontic restorations

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Authors' contribution

I carried out the experimental work, interpreted data, designed and wrote the manuscript of the paper. Niazi S.A participated in microbiological testing. All authors helped to evaluate and edit the manuscript.

Abstract

Objectives: The purpose of this study was to incorporate EgMA, an antibacterial monomer into two commercial dental adhesives systems for their application in endodontic restoration with the aim to disinfect the root canal space before curing and to inhibit bacterial growth on their surfaces after being cured.

Methods: EgMA monomer was added at 20% wt. into the formulation of the single-component self-etch, Clearfil Universal Bond™ (CUB) and into the catalyst and the adhesive components of the total-etch Adper Scotchbond-multipurpose™ (SBMP)

adhesive systems. The degree of conversion (DC) was calculated from FTIR spectra, glass transition temperature (*T*g) determined by DSC, water sorption and solubility were measured gravimetrically, and surface free energy (SFE) via contact angle measurements. The bonding performance to coronal and middle root canal dentine was assessed through push-out bond strength after filling the canals with a composite core material and the surface integrity was observed using SEM and confocal laser scanning microscope (CLSM). The standard agar diffusion test (ADT) was used to identify the sensitivity of three endodontically pathogenic bacteria, *E. faecalis, S. mutans and P. acnes* to uncured EgMA modified adhesives. Multispecies biofilm model from these strains was grown on the disc surface of cured adhesives and investigated using quantitative microbial culture and CLSM with live/dead staining. MTT assay was also used to determine the cytotoxicity of these adhesives.

Results: The incorporation of EgMA lowered polymerisation exotherm and enhanced the hydrophobic character of these adhesives, without changing the DC and *T*g in comparison to the controls (without EgMA). The total push-out bond strengths of the EgMA-containing adhesives were not significantly different from those of the controls ($P>0.05$). The modification of self-etch adhesive system enhanced the bond strength in the middle region of the roots canal. SEM of debonded specimens and CLSM examination showed the integrity of the resin-dentine interfaces. For all three bacteria tested, the sizes of the inhibition zones produced by uncured EgMA modified adhesives were significantly greater ($P<0.05$) than those of the controls. The results of biofilm inhibition tests showed less CFU for total bacteria on bonding agents with EgMA compared to the control materials ($P<0.05$). The modification at 20% monomer concertation had no adverse effects on cytocompatibility of both adhesives tested.

Significance: The inclusion of EgMA endows dental adhesives with effective antibacterial effects without influencing their curing properties, bonding ability to root canal dentine, and cytotoxicity against human gingival fibroblasts, indicating the usefulness of their application in endodontic restorations.

5.1.1 Introduction

Bonding of posts to root canal dentine is still a challenge due to the reduced number of dentinal tubules in the apical third of the root (Mjor *et al.* 2001), limited access and visibility. Furthermore, the large configuration factor (C-factor) of the endodontic cavity results in a high contraction stress, that can exceed the bond strength, increasing the risk of voids and microgaps within the cement interface with subsequent bonding failure and microleakage (Tay *et al.* 2005). Microleakage can cause new bacterial invasion of the root canal space also, complete removal of bacteria from the root canal system following the cleaning and shaping of the root canal is at present impossible to achieve (Vieira *et al.* 2012). Residual bacteria often remain in the tubules, which may repopulate the root canal and jeopardise clinical performance and longevity of the endodontic restoration. Therefore, imparting an antibacterial function to dental restorative materials, and in particular to the dental adhesives as they directly contact tooth structure and infiltrates into dentinal tubules is expected to disinfect the cavity, lowering the risk of reinfection and secondary caries.

Several attempts to produce dental adhesives with antibacterial activity have been attempted either by the addition of soluble antimicrobial agents, such as chlorhexidine, or immobilisation of antibacterial components in the resin matrix (Imazato 2003). However, the release of antibacterial agent could cause an adverse effect on mechanical properties, toxicity and short-term antibacterial effectiveness whilst, the immobilisation of antimicrobial agents prevents or reduces colonisation of contacted bacteria without leaching out from the material, resulting in long-lasting antibacterial activity without adverse effects on mechanical properties and bonding characteristics (Rojo and Deb 2015). A number of ionic mono and di-methacrylate monomers containing quaternary ammonium groups have been incorporated into existing dental dimethacrylate-based monomers demonstrating bactericidal activity. For instance, Clearfil protect bond, which contains methacryloyloxydodecylpyridinium bromide (MDPB), and dental adhesives with methacryloxylethyl cetyl dimethyl ammonium chloride (DMAE-CB) have been found to

exhibit an inhibitory effect on the growth of *S. mutans* (Li *et al.* 2009). However, some of the quaternary ammonium based monomers exhibit miscibility problems with hydrophobic dimethacrylates (Antonucci *et al.* 2012). In addition, incorporation of these monomers at high concentrations to obtain reliable antibacterial effects result in adverse effects on mechanical properties and unwanted release of the monomers into the surrounding tissues (Ebi *et al.* 2001).

Eugenol is a well-known antimicrobial essential oil, which is used in combination with zinc oxide in different dental applications such as temporary filling materials and root canal sealers and is very effective against a range of oral bacteria (Kaplan *et al.* 1999; Lai *et al.* 2001). The main disadvantage of eugenol-containing materials is the fact that they inhibit the polymerisation reaction of methacrylate resins due to remaining free eugenol. Eugenyl methacrylate (EgMA) is an eugenol derivative that is able to copolymerise with other methacrylate monomers after curing and immobilises the antibacterial eugenol moieties in the polymer backbone without the inhibitory effect characteristic of the phenol group (Rojo *et al.* 2006). The authors have previously reported that the incorporation of an antibacterial monomer EgMA was effective in providing resin composite materials with intrinsically antibacterial activity against a range of oral bacteria commonly associated with the failure of coronal and endodontic restorations (Almaroof *et al.* 2016b). This effect based on the strong antibacterial activity of EgMA monomer (Rojo *et al.* 2006; Rojo *et al.* 2008a). In addition, the immobilisation of eugenol is advantageous as it avoids the migration of this molecule into the surrounding tissues and improves its hydrolytic stability.

Thus, the aim of the study was to investigate the efficacy of the modified dental adhesives via the inclusion of the eugenol methacrylate derivative. The influence of this monomer on curing properties, *T*g, wettability, water sorption, bonding ability and cytotoxicity of these modified bonding agents are reported.

5.1.2 Materials and methods

5.1.2.1 EgMA incorporation into bonding agents

Two commercial adhesives, Clearfil Universal Bond™ (CUB) and Adper Scotchbond™ multi-purpose plus (SBMP) adhesives were used in this study as parent bonding systems to test the effects of incorporation of the antibacterial monomer. Their manufacturers and chemical composition are presented in Table 5.1-1.

Table 5.1-1 The chemical compositions of bonding agents tested in this study

Boding agent	Manufacturers (patch number)	Code	Composition
Clearfil Universal Bond™ (Self-etch)	Kuraray, Tokyo, Japan (1562R041R)	CUB	MDP, Bis-GMA, HEMA, hydrophilic aliphatic dimethacrylate, colloidal silica, silane coupling agent, CQ, ethanol, water
Modified Clearfil Universal Bond™		Mod.CUB	CBU + 20 wt.% stock solution of EgMA*
Adper Scotchbond™ multi-purpose plus (Total-etch)	3M ESPE, St. Paul, MN, USA (N662538)	SBMP	Etchant: 35% Phosphoric acid gel Activator: Ethyl alcohol, sodium benzenesulfinate Primer: Water, HEMA, copolymer of acrylic, itaconic acids Adhesive: Bis-GMA, HEMA, Tertiary amines, photi-initiator Catalyst: Bis-GMA, HEMA, Benzoyl peroxide
Modified Adper Scotchbond™ multi-purpose plus		Mod.SBMP	SBMP Etchant SBMP Activator SBMP Primer SBMP Adhesive + 20 wt.% stock solution of EgMA* SBMP Catalyst + 20 wt.% stock solution of EgMA*

Abbreviations: MDP=10-Methacryloyloxydecyl dihydrogen phosphate; Bis-GMA = 2, 2-Bis [4- (2-hydroxy-3 methacryloyloxypropyl)-phenyl] propane; HEMA = hydroxyethyl-methacrylate; EgMA = eugenyl methacrylate; CQ = camphoroquinone ; EDAB = 4-(dimethylamino) benzoate.
*Stock solution = [EgMA monomer + CQ / EDAB (0.5 wt./0.5wt)]

EgMA monomer was synthesised via a method reported previously by Rojo *et al* (Rojo *et al.* 2006). A stock solution of EgMA monomer and CQ / EDAB (0.5wt/0.5wt) both from Sigma-Aldrich (Company Ltd, Dorset, UK) was added at 20% by weight into the formulation of single-component CUB and into the adhesive/catalyst components of SBMP to prepare two modified experimental adhesives, designated respectively, as Mod.CUB and Mod.SBMP (Table 5.1-1). The selection of this percentage was based on pilot study that showed that the addition of 20 wt.% EgMA into Bis-GMA/HEMA (70/30 wt.%) blend, a commonly used dental adhesive resin formulation, had no adverse effects on degree of monomer conversion and *T*g of the polymers.

5.1.2.2 Specimen preparation

For the solvated one-bottle, CUB control and modified CUB adhesives, the solvent was evaporated under reduced pressure in a dark container until the resin reached a constant mass as solvent evaporation was assumed to be complete and then carefully placed into different moulds. For total-etch SBMP control and modified SBMP adhesives, the activator and primer were first smeared on moulds, dried with a gentle stream of air, then equal masses from adhesive and catalyst components were mixed and applied. For water sorption, solubility, surface contact angle, cytotoxicity and biofilm inhibition tests, resin discs of each material were produced in Teflon mould (10 mm diameter, 1 mm thick). After filling the mould, the discs were covered with glass slides to exclude atmospheric oxygen, and then cured by visible light for 40 s, using a dental curing unit (Optilux, Demetron Res Crop, Danbury, USA) with an irradiance of 600 mW.cm^{-2}. After removing the specimen from the mould, light-curing was repeated on the opposite surface for another 40 s.

5.1.2.3 Degree of conversion

The degree of conversion of each adhesive was analysed before and after cure using FTIR spectroscopy (ATR accessory, Spectrum one, Perkin Elmer, Waltham, MA, USA). The spectra of the polymer were obtained by light-curing one drop of each adhesive between two translucent Mylar strips, pressed to produce a very thin film. Five cured specimens of each group were tested 10 min after curing and after 24 h storage at 37 °C. The degree of cure was determined using equation 1

$$\text{Degree of conversion (\%)} = \left[1 - \frac{(A1637/A1608)\ polymer)}{(A1637/A1608)\ monomer)}\right] \times 100 \qquad (1)$$

Where A1637 and A1608 correspond to the absorbance of the aliphatic $\upsilon_{C=C}$ peak registered at 1637 cm^{-1} and to the aromatic $\upsilon_{C=C}$ peak registered at 1608 cm^{-1} respectively before and after polymerisation.

5.1.2.4 Curing temperature

A thermocouple (1.3 mm diameter) fitted out to a high-sensitivity temperature recorder (KM1242, Herts, UK) was used to measure the polymerisation temperature. The wire was placed centrally in a cylindrical Teflon mould (4 mm diameter, 12 mm depth) filled with each adhesive material and its stripped ends were levelled with the material's surface to be irradiated. The materials were polymerised for 40 s from one side and the maximum temperature was reported during the polymerisation cycle. Five measurements were done for each tested material at 23 °C.

5.1.2.5 Thermal properties

Differential scanning calorimetry (DSC) was carried out using a Perkin Elmer machine (Waltham, MA, USA) to determine the glass transition temperature of the cured adhesives. Samples of about 10 mg were heated from 0 °C to 230 °C at the rate of 20 °C/min in an inert N_2 atmosphere. Three samples from each formulation were tested.

5.1.2.6 Measurement of contact angle and surface free energy (SFE)

The contact angle θ and SFE (Y_s) were evaluated on bonding surface discs using the sessile drop method as described in our previous study (Almaroof *et al.* 2016b).

5.1.2.7 Water sorption and solubility

Water sorption and solubility were determined according to the ISO specification 4049. Five resin discs (10 mm diameter, 1 mm thick) of each adhesive material were immersed in 10 ml distilled water and weighted at noted interval during the 28 days immersion period. The mass change percentage was calculated using equation 2:

$$\text{Water uptake} = (Ms_{(t)} - Mi) / Mi \times 100 \qquad (2)$$

where, Mi is the initial mass of the specimen and Ms is the mass of saturated specimen at the end of the immersion period.

The specimens were dry-stored again at 37 °C and reweighed using approximately the same time intervals until a constant dry mass (Md) was obtained.

Sorption (SR) and solubility (SL) in µg/mm³ were calculated based on the percentage of mass gain or loss during the sorption and desorption cycles using the following equations:

$$SR = Ms - Md / V \quad (3)$$

$$SL = Mi - Md / V \quad (4)$$

5.1.2.8 Push-out bond strength test

Thirty-six human single-rooted premolars extracted after obtaining an informed consent of the patients and following a protocol approved by an institutional review board were used in this study (Research Ethics Committee reference number 14/LO/0123). All teeth were stored at 4 °C in distilled water and used within one month. Teeth were randomly and equally assigned to 4 groups based on the adhesive materials used in this study (n=9), three specimens from each group were reserved for confocal microscopy analysis. The crown was sectioned at the cemento-enamel junction using a low speed; water-cooled diamond saw microtome (Isomet 1000, Buehler, Lake Bluff, IL, USA). The teeth were endodontically treated with nickel-titanium rotary instruments (Protaper; Dentsply Maillefer, Ballaigues, Switzerland) and 1% sodium hypochlorite irrigation. The canal was filled with gutta-percha and calcium hydroxide endodontic sealer (Sealapex, Kerr, SpA, Salerno, Italy) using the lateral condensation technique. The prepared roots were mounted vertically in acrylic resin block using an aligning device. After 24 h storage at 37 °C in relative humidity, the first 8 mm of the canal was shaped with a cylindrical flat end diamond bur (Komet 837/016, Lemgo, Germany) so that a standardised cavity of 2 mm in diameter was prepared in the coronal and middle portion of the root canal.

The dental adhesives were applied in accordance with the manufacturer's instructions. For the CUB control and modified CUB self-etch adhesives, the bonding agent was applied to the entire root canal with the applicator brush and rubbed for 10 s, then dried with a gentle air blow for 10 s and light-cured for 40 s using dental light-curing unit at 600 mW cm^{-2} (Optilux, Demetron Res Crop, Danbury, USA).

For the SBMP control and modified SBMP total-etch adhesives, the root dentine was acid-etched with 35% phosphoric acid gel for 15 s, rinsed for 20 s with water and dried with absorbent paper points (Dentsply/Maillefer, Petrópolis, RJ, Brazil). A layer of activator was applied into the root canal using disposable brushes, followed by air-drying for 5 s. The primer was applied and air-dried for 5 s. The adhesive and catalyst were mixed and applied with a fresh disposable brush and light-cured for 40 s. A dual-cure composite core (Clearfil™DC Core plus, Kuraray, Japan) was injected into the post-space and light-cured for 60 s. The root segment was then placed in individually labelled containers in relative humidity at 37 °C. After 24 h, the bonded roots were transversely sectioned to create 1.5 mm thick root slices, the thickness was verified using a digital electronic calliper, the top root slices were discarded to avoid the influence of excess material, producing four root slices from each root (2 coronal and 2 middle) for subsequent push-out bond strength tests. Each slice was marked with a permanent marker on its coronal aspect and sufficiently supported by a stainless steel jig with clearance for the dislodged core material. The push-out force was applied in an apical-coronal direction using a cylindrical plunger with a diameter of 1.8 mm attached to a universal testing machine (Instron model 5569A-Series Dual Column, High Wycombe, UK) at a crosshead speed of 0.5 mm/min until failure. The maximum load at failure was recorded in Newton (N) and was converted to MPa by dividing the applied load by the bonded area, using the following equation:

Push-out bond strength = force/ π x diameter x thickness (5)

The modes of failure were examined visually using a stereomicroscope (WILD M32; Heerbrugg, Switzerland) at x30 and classified as adhesive failure between core material and dentine, cohesive failure with complete dentine or core material cohesive failure and mixed failure with partial interfacial adhesive failure with the presence of core/dentine cohesive failure. Furthermore, four representative debonded specimens per group that failed in mixed or adhesive modes were selected to analyse the ultramorphology of the fractured surface with SEM. The specimens were dried overnight, mounted on aluminium

stubs with carbon cement, sputter-coated with gold and observed with a scanning electron microscope (SEM, Hitachi High Technologies, S-3500N) at an accelerating voltage of 10 KeV and increasing magnifications.

5.1.2.9 Confocal laser scanning microscopy (CLSM)-Interface evaluation

For this analysis, 0.1 wt.% fluorescein dye (Sigma-Aldrich, UK) was added to the single bottle component of CUB adhesives (control and modified) and to the adhesive and catalyst bottles of SBMP (control and modified) while, 0.1 wt.% Rhodamine B (Rh B: Sigma- Aldrich, UK) was added into the primer bottle of Adper SBMP adhesives.

A further three specimens from each group were bonded, as previously described, with these labelled adhesive systems and employed for the confocal microscopy analysis. After 24 h storage in 100% relative humidity, the specimens were longitudinally sectioned into two halves and polished using wet SiC abrasive papers of ascending grit #600 to #2500 (Versocit; Struers) with final ultra-sonication treatment in a distilled water bath for 5 min. The microscopy examination was performed using a confocal laser scanning microscope (Leica SP2 CLSM; Leica, Heidelberg, Germany) equipped with a 63x/1.4 NA oil-immersion lens and using 488-nm argon/helium (fluorescein excitation) or 568-nm krypton (rhodamine excitation) laser illumination. The entire resin-dentine interface was completely investigated and three representative images of the most common distinguishing characteristics detected in each specimen were captured. All images were further reconstructed with Image J software.

5.1.2.10 Antibacterial assays

5.1.2.10.1 Agar Diffusion Test (ADT)

The antibacterial activity of uncured adhesive resins was evaluated by agar diffusion test against *E. faecalis, S. mutans* and *P. acnes*. The bacteria were revived from - 80 °C and plated on FAA plates (Fastidious Anaerobe Agar with 5% Horse Blood- Lab M, UK).

Bacterial test suspensions with a concentration of 6×10^5 colony forming units (CFU)/mL were prepared from the pre-cultures. An aliquot of 150 µL of the bacterial suspension was spread evenly throughout the FAA agar plate using sterile swabs. Under aseptic conditions, a 20 µL portion of each bonding agent was absorbed onto sterile paper discs (6 mm diameter, 1.5 mm thick, Schleicher & Schuell, Germany) and placed on the inoculated agar surface (n=3). Pure eugenol was used as positive control. After anaerobic incubation of the plates at 37 °C for 48 h, the inhibition zones produced around the paper discs were measured [Outer diameter of inhibition zone - paper disk diameter)/2].

5.1.2.10.2 Biofilm inhibition test

Three-species biofilms composed of *E. faecalis, S. mutans and P. acnes* were grown on cured resin adhesives discs to investigate their capacity to reduce or inhibit colony formation by these bacterial species. To establish the biofilm, the bacterial strains were cultured anaerobically at 37 °C in MACS-MG-1000-anaerobic workstation (80% nitrogen, 10% hydrogen, 10% carbon dioxide) on Fastidious Anaerobe Agar supplemented with 5% defibrinated horse blood (FAA, Lab M, Heywood, UK). An individual starter culture of each bacterial strain was transferred into 3 mL of modified fluid universal medium (mFUM) and incubated anaerobically at 37 °C for 3 h. The absorbance was adjusted with fresh mFUM to 0.5 at 540 nm to obtain 10^7 cells mL-1 using Labsystems iEMS Reader (MF, Basingstoke, UK).

All discs (n= 6, for each adhesive) were soaked in distilled water at 37 ° C for 24 h to remove unpolymerised monomers and then sterilised by wiping with 70% ethanol in water and were exposed to UV radiation for 30 min. Discs were placed in 1 mL of mFUM contained in 24-well tray and pre-reduced in the anaerobic workstation. The discs were then seeded with 400 µL (4×10^6 cells) of each of the three starter cultures. The biofilms were grown anaerobically with regular medium change every 24 h for the first 7 days. In order to nutritionally starve the biofilms, they were further grown anaerobically for another

7 days without medium change following the protocol previously mention by Niazi *et al*. (Niazi *et al.* 2014).

To enumerate the numbers of bacteria in the biofilms, each disc was placed in 1 mL of BHI (Brain-Heart Infusion Broth, Lab M) and vortex for 1 min to disperse the biofilm from the surface of the disc. After serial dilution in BHI, aliquots (100 µL) were plated onto duplicate FAA plates and incubated anaerobically for 7 days and colonies were counted.

Two discs from each group were gently washed twice with PBS to remove non-adherent cells and stained with Live/Dead BacLight bacterial viability kit (Invitrogen, Paisley, UK) and washed again before visualiSation under a Leica SP2 confocal laser scanning microscope (CSLM). The quadrants of the biofilm on resin disc were demarcated by 4 marks made at the corners of the glass bottom of the 35 mm diameter Petri dishes (SLS, UK) by using a permanent marker. Biofilm structure was examined by three different areas in each quadrant of the biofilm. The mean percentages of dead (red) and live (green) biovolumes were analysed using *bio*Image_L (Chávez de Paz 2009).

5.1.2.11 Cytotoxicity

MTT (methyl tetrazolium) assay was used to evaluate the cytotoxicity of adhesives with human gingival fibroblast (P8, ScienCell™ Res. Lab., UK) at 24 h and 48 h according to the International Standard ISO 10993-5. Adhesives eluents were obtained by immersing sterile disc samples in 3 ml of fibroblast medium (500 ml basal medium, 10 ml fetal bovine serum, 5 ml of fibroblast growth supplement and 5 ml of penicillin/streptomycin solution, ScienCell™, UK) within bijou vials, which were then placed onto a roller at room temperature. The supernatants were collected at 24 and 72 h time points and refrigerated at -20°C to be used for cytotoxicity measurements.

HGF cells were cultured at 37 °C humidified atmosphere with 5% CO_2 to reach about 80% confluent, trypsinised and then seeded on a 96-well plate (100 µL / well) at a density of 1 x 10^4 cells/well. The cells were incubated at 37 °C, 5% CO_2 for 24 h to allow for cell to attach and acclimatisation prior to addition of the test eluents. After 24 h, the fibroblast

media were removed from both plates and replaced with 100 µl of the leached eluents from adhesives. Untreated cells served as a negative control while positive control cells were treated with 10% v/v ethanol solution. Each group consisted of five replicate wells. Then the plates were incubated for 24 h or 48 h (exposure times), after which the test eluents were removed and replaced with 100 µL of MTT (5 mg/mL PBS) for 4 h. MTT solution was then removed and 100 µL dimethyl sulfoxide (DMSO) was added to each well. The plate was shaken for 5 min and the absorbance of the purple coloured solution was measured using a UV–visible spectrophotometer plate reader at wavelength 570 nm (Opsys MR, Dynex Technologies, Chantilly, VA, USA). Relative cell viability is then expressed as a percentage of untreated negative control reading. Each experiment was done in duplicates.

5.1.2.12 Statistical analysis

After analysing the normality of data distribution (Kolmogorov-Smirnov test), a Mann-Whitney (nonparametric) test or Independent *t*-test (for normally distributed values) was used to determine the effects of EgMA monomer addition on properties of commercial parent adhesives tested. A one-way (ANOVA) and Tukey's post-hoc test were also employed for the statistical evaluation of ADT and cytotoxicity data. In all tests, the level of significance was set at $P<0.05$.

5.1.3 Results

5.1.3.1 Curing parameters

The initial degrees of cure for both EgMA modified adhesives were significantly lower than their corresponding control [Table 5.1-2]. However, DC of these bonding agents increased when measured at 24 h post curing, reporting values which were not statistically different from the controls ($P>0.05$). The addition of EgMA into the formulations of bonding agents significantly lowered the polymerisation exotherm ($P<0.05$).

Table 5.1-2 Degree of conversion (10 minutes and 24 h post curing) and polymerisation exotherm of the studied bonding agents (mean [SD], n=5)

Bonding agent	DC in % 10 min post cure [SD]	DC in % 24 h storage at 37 °C [SD]	Polymerisation exotherm °C [SD]
CUB	91 [0.9]	95 [1.7]	66.8 [1.8]
Mod.CUB	88 [0.8]*	92 [1.9]	54.0 [1.8]*
SBMP	74 [0.5]	76 [1.0]	98.9 [1.7]
Mod.SBMP	66 [1.0]*	74 [1.5]	86.6 [1.5]*

*** Differences were statistically significant with respect to the corresponding unmodified bonding agent (*P*<0.05)**

5.1.3.2 Glass transition temperature (*T*g)

The DSC thermograms for the bonding agents tested are shown in Fig. 5.1-1. *T*g values of CUB and Mod.CUB were 78.3 ± 1.1 °C and 77.1 ± 0.4 °C respectively, for SBMP and Mod.SBMP were 68.5 ± 0.8 °C and 67.8 ± 0.8 °C respectively. Both EgMA modified bonding agents exhibited comparable *T*g values to their corresponding controls which were statistically not significant (*P*>0.05).

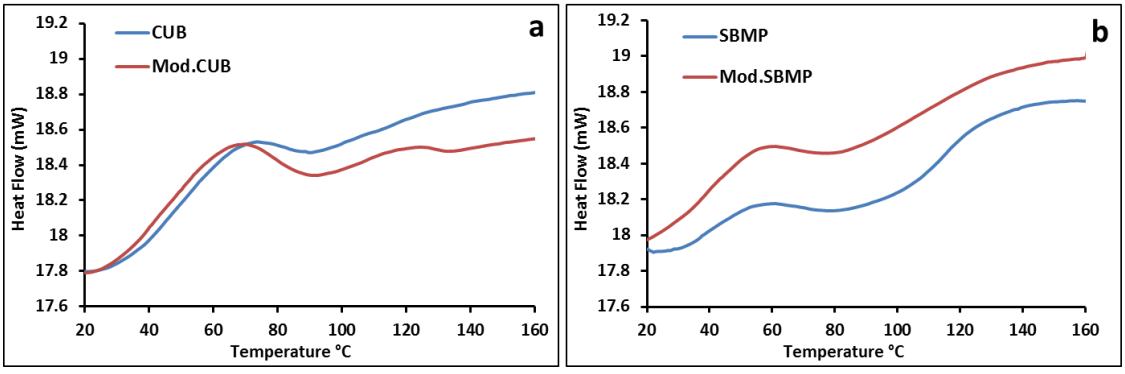

Figure 5.1-1 DSC representative curves of (a) CUB and Mod.CUB adhesives and (b) SBMP and Mod.SBMP adhesives

5.1.3.3 Water sorption, solubility and wettability

The percentage water uptake during the 28-days of immersion is presented in Fig. 5.1-2.

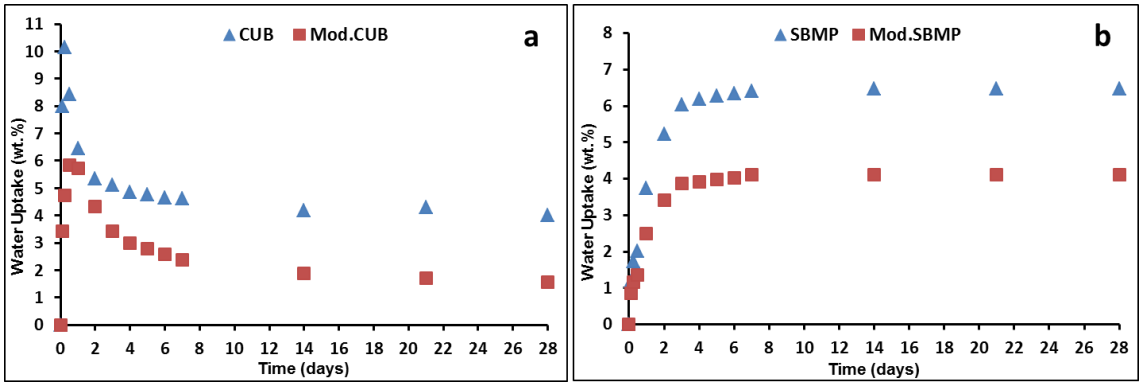

Figure 5.1-2 Mass changes in percentage of the bonding agents during immersion in water over 28 days

All adhesives showed the greatest mass change within the 1st day of storage in water. After that period, for both CUB and Mod.CUB, a constant decrease in mass was observed until equilibrium was reached, which occurred between the 2nd and 3rd day. Conversely, SBMP and Mod.SBMP showed a continued increase of mass until equilibrium. Both eugenyl containing bonding agents showed a reduction of water sorption capacity.

Contact angle (Θ) values, surface free energy (γ_s), water sorption and solubility for all bonding agents tested are summarised in Table 5.1-3. The addition of EgMA into the bonding formulation significantly increased the contact angle measurements with both tested liquids indicating the higher hydrophobicity of bonding surfaces. Accordingly, the surface free energy calculated decreased significantly. A significant reduction in SR and SL of Mod.CUB and SR of Mod.SBMP bond was also observed (P<0.05), however, no significant difference in SL of Mod.SBMP bond was found in comparison to the control.

Table 5.1-3 Contact angle, solid surface free energy, water sorption (SR) and solubility (SL) for the bonding agents studied

Composites	Θ (H_2O) [S.D.^{n}]	Θ (CH_2I_2) [S.D.^{n}]	Y_s (mN/m) [S.D.^{n}]	SR (µg/mm^3) [S.D.^{m}]	SL (µg/mm^3) [S.D.^{m}]
CUB	58.5 [3.7]	38.7 [2.2]	53.7 [2.0]	126.8 [2.1]	89.2 [1.4]
Mod. CUB	62.8 [4.0]*	41.5 [1.8]*	50.5 [2.0]*	95.1 [0.2]*	79.2 [0.6]*
SBMP	64.4 [5.7]	42.2 [5.8]	49.6 [3.2]	80.8 [1.6]	3.8 [0.5]
Mod. SBMP	69.1 [2.4]*	46.2 [4.8]*	45.6 [1.6]*	51.4 [0.4]*	3.7 [0.9]

S.D.= standard deviation, n = 10; m = 5; Θ (H_2O)= water contact angle; Θ (CH_2I_2) = methylene iodide contact angle. * Differences were statistically significant with respect to the corresponding unmodified bonding agent (P<0.05)

5.1.3.4 Push-out bond strength and SEM analysis of debonded specimens

The results of the push-out bond test and the percentages of the failure modes are shown in Table 5.1-4. The total bond strengths achieved by Mod.CUB (self-etch) and Mod.SBMP (total-etch) adhesives were statistically not significant than that of their corresponding controls (*t*-test, P>0.05). The failure analysis was in accordance with the push-out bond strength results of both adhesive systems, the specimens failed mainly in adhesive and mixed mode; however, some specimens exhibited composite cohesive failure. Mod.CUB bond showed more cohesive failures compared to the controls.

When comparing the bond strength achieved at root level using the two bonding agents (control and modified) for each adhesive system on trial, the independent *t*-test showed that the bonding ability of Mod.CUB in the middle region of the roots was significantly greater than that of CUB control (P<0.05). In contrast, there was no such difference observed in the coronal region of the roots (P> 0.05). The bonding ability of SBMB and Mod.SBMP were comparable in both regions of the roots.

Table 5.1-4 Comparison of the mean push-out bond strength (MPa) values and the analysis of failure modes

Bonding agents	Bond strength values (mean [SD])			Total failure mode (%)		
	Regional BS (n=12)		Total BS (n=24)	Adhesive	Mixed	Cohesive
	Coronal	Middle				
CUB	11.3 [2.3]	6.2 [2.0]	8.8 [3.3]	62	30	8
Mod.CUB	12.4 [1.5]	8.2 [2.4]*	10.3 [2.9]	54	34	12
SBMP	12.1 [2.0]	6.9 [0.7]	9.5 [3.0]	54	38	8
Mod.SBMP	11.6 [2.1]	6.2 [1.1]	8.94 [3.2]	58	34	8

*** Differences were statistically significant with respect to the corresponding unmodified bonding agent (*P*< 0.05)**

SEM evaluation of debonded specimens that failed in adhesive or mixed modes is shown in Fig. 5.1-3. A resin-hybridised dentine surface predominantly covered by residual resin was observed, however, some exposed dentinal tubules were evident in the middle region of the roots for the adhesives tested. The mid-sections of the roots adhesively bonded with the modified CUB showed that resin tags formed on most dentinal tubules in comparison with the control CUB adhesive, which also explained the higher bond strengths obtained in this region (Fig. 5.1-3, B2 and A2).

The modified SBMP adhesive showed features, which were comparable to that of its corresponding control without EgMA in coronal and middle specimens, although few resin tags inside the tubules were often detected on the surface of the middle specimens which are characterised by more exposed dentinal tubules (Fig. 5.1-3 D2 and C2).

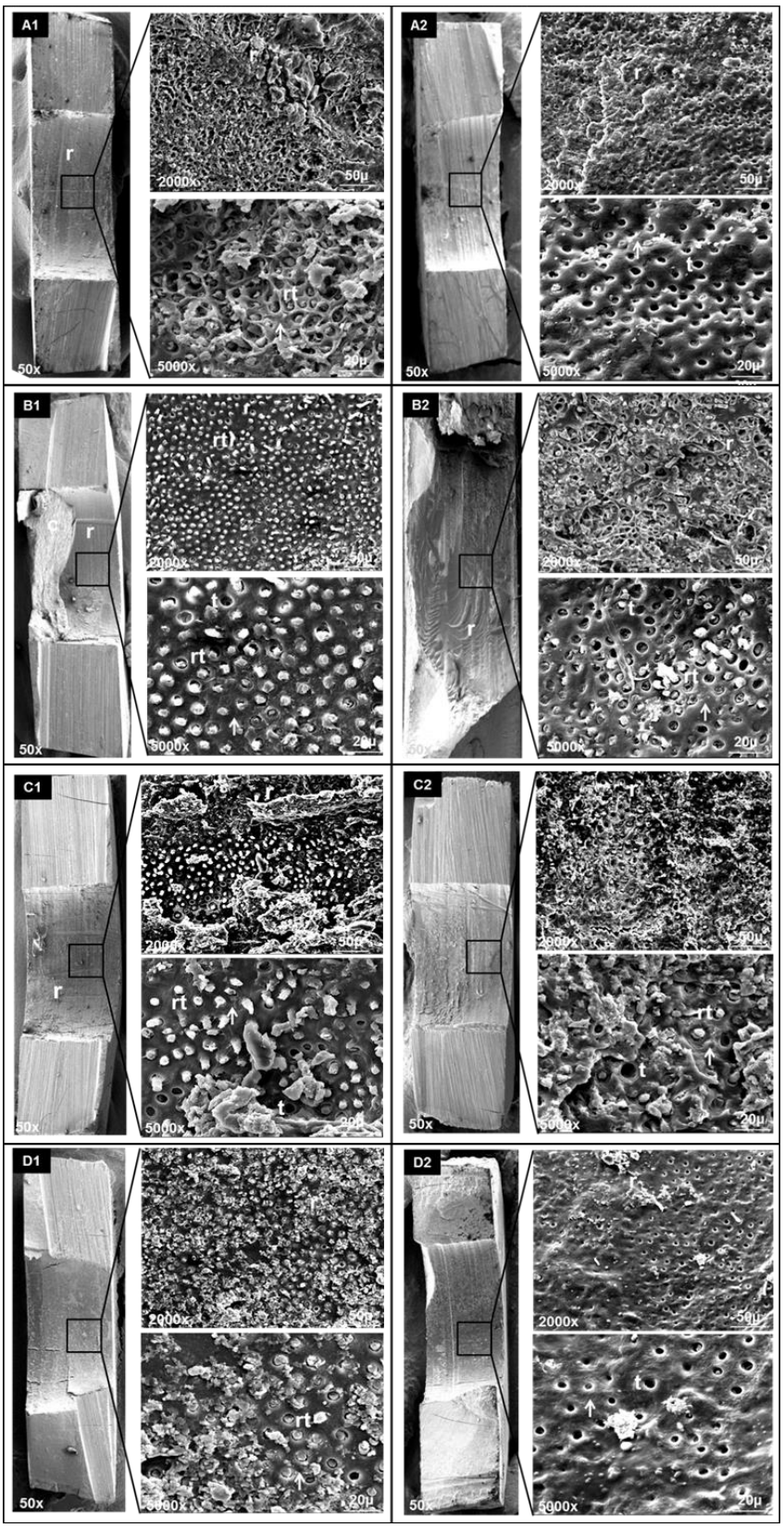

Figure 5.1-3 SEM images of debonded specimens created using the modified adhesives tested in comparison with their control, taken from coronal (1) and middle (2) regions at different magnifications showing the features of failure in the adhesive layer

(A1) and (A2) are representative images of the coronal and middle sections respectively, created with unmodified CUB adhesive, which failed in adhesive mode leaving a residue of resin adhesive (r). At higher magnification shows that the residual resin blocked the dentinal tubules completely or partially and exposed dentinal tubules (t) which were more evident in the middle region (A2). (B1) and (B2), representative images of coronal and middle sections created with Mod.CUB adhesive, (B1) coronal specimen failed in mixed mode leaving a presence of residual resin adhesive (r) and composites (c), (B2) mid-section failed in adhesive mode leaving residual resin adhesive. At higher magnification, some exposed dentinal tubules (t) are visible but most remained obliterated by resin tags (rt), of which some tags were protruding from the debonded surface and fewer number of exposed dentinal tubules were observed in the middle region in comparison with CUB adhesive. For both adhesives, a well-formed hybrid layer was present on dentine surface (arrows). (C1) and (C2) show representative images of the coronal and middle sections respectively, created with unmodified SBMP adhesive, which failed in adhesive mode showing an important presence of residual resin (r) and exposed dentine with a well-formed hybrid layer (arrows). At higher magnification, it is possible to observe most of the dentinal tubules were obliterated with resin tags (rt) with few exposed tubules (t). Images from modified SBMP adhesive (D1) coronal and (D2) middle specimens (in adhesive mode) showed comparable features after failure. It is possible to see also a well-formed hybrid layer (arrow) and residual resin (r). A few resin tags inside the dentinal tubules were observed on the dentine surface of the middle specimen (D2). r, resin; t, dentinal tubules; rt, resin tags; c, composite.

5.1.3.5 CLSM-Interface evaluation

Similarly to their corresponding controls (unmodified adhesives), the CLSM assessment showed that both EgMA modified self-etch CUB and total-etch SBMP adhesives were able to create a resin diffusion zone within the coronal and middle root dentine, forming a clear hybrid layer (approximate thickness 7-9 µm) located underneath a thick adhesive layer. The presence of resin tags is also evident in both adhesives and regions (Fig 5.1-4).

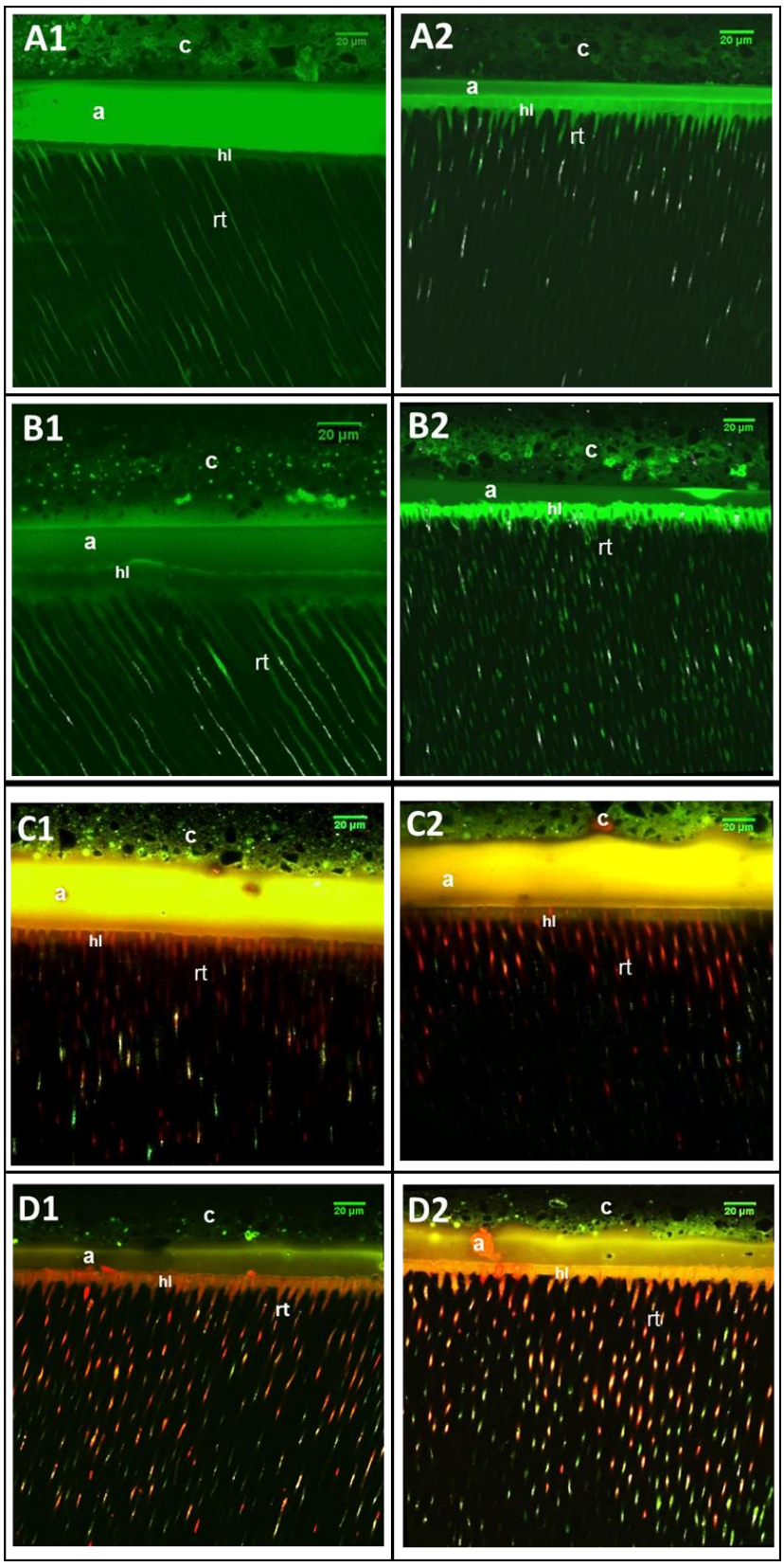

Figure 5.1-4 Confocal laser scanning microscopy (CLSM) images showing the interface of the resin–dentine created using the four adhesives tested from coronal (1) and middle (2) regions of the root after 24 h storage in 100% relative humidity

(A) and (B) CLSM images of the resin–dentine interfaces, taken from the coronal (1) and middle (2) regions of the roots, created with the CUB and Mod.CUB adhesives, respectively, which were labelled with fluorescein dye (green). CLSM (fluorescence/reflection mode) images show that both control unmodified and EgMA modified CUB adhesives were able to diffuse into root coronal (A1 and B1, respectively) and middle (A2 and B2, respectively) dentine, creating a gap-free interface and a clear hybrid layer (approximate thickness 7-9 μm) located underneath a thick adhesive layer and longer resin tags. (C) and (D) CLSM images of the resin–dentine interfaces, taken from the coronal (1) and middle (2) regions of the roots, created with the SBMP and Mod.SBMP adhesive systems, respectively. The primer was labelled with rhodamine B and the adhesive was labelled with fluorescein, showing red and green fluorescence colours, respectively. CLSM (fluorescence/reflection mode) composite images demonstrate an orange colour interface, which corresponded to the mixture between the primer and the adhesive components, indicating the ability of the adhesive components in both control and EgMA modified SBMP systems to diffuse into acid-etched root coronal (C1 and D1, respectively) and middle (C2 and D2, respectively) dentine, creating a gap-free interface and a clear hybrid layer (approximate thickness 7-9 μm). Thicker adhesive layers were observed in control adhesive than in modified SBMP adhesive. Resin tags are also seen in both adhesives and regions. a, adhesive layer; c, composite; hl, hybrid layer; rt, resin tags.

5.1.3.6 Antibacterial activity

ADT results (Table 5.1-5) showed that pure eugenol and both EgMA modified resin adhesives produced clear inhibition zones against all three bacteria tested, exhibiting statistically significant differences between them following the order *P. acnes* > *S. mutans* >*E. faecalis* (P<0.05). The unmodified CUB exhibited some inhibition against *P. acnes* and *E. faecalis*, whilst the unmodified SBMP produced inhibition against *P. acnes* only. However, the zone of inhibition observed for EgMA modified adhesives were significantly higher than those obtained with the controls. The inhibition of bacterial growth noted with Mod.CUB against all species tested tended to be greater than the inhibition recorded for Mod.SBMP, however, the *t*-test showed significance in *E. faecalis* species only.

Table 5.1-5 Size of inhibition zones produced against *S. mutans, E. faecalis and P. acnes*

Group	Inhibition zone size in mm, mean [SD] of three replicates		
	S. mutans	*E. faecalis*	*P. acnes*
Eugenol	**13.4 [0.6]**	**7.6 [0.6]**	**21.0 [1.2]**
CUB	**0**	**2.5 [0.5]**	**6.4 [0.6]**
Mod.CUB	**16.3 [1.1]***	**9.5 [0.5]***	**21.0 [1.1]***
SBMP	**0**	**0**	**5.8 [0.7]**
Mod.SBMP	**14.8 [0.7]***	**6.5 [1.3]***	**19.1 [1.0]***

*** Differences were statistically significant with respect to the corresponding unmodified bonding agent (*P*<0.05)**

The results of biofilm inhibition test are shown in Fig. 5.1-5 The means number (± SD) of bacteria in 14-d biofilm grown on the surface of cured resin discs of the Mod.CUB (7.20 ± 0.14) and Mod.SBMP (7.74 ± 0.21) adhesives, as log_{10} CFU, were significantly lower than their corresponding controls CUB (8.07 ± 0.05) and SBMP (8.9 ± 0.13) adhesives. EgMA had a significant effect on the recovery of bacteria.

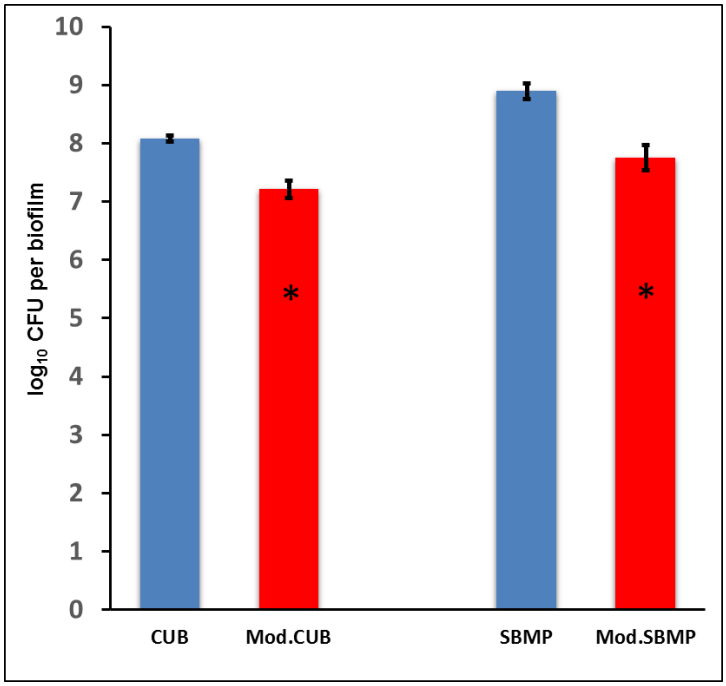

Figure 5.1-5 Colony-forming unit (CFU) counts for total microorganisms in 14-d biofilm on cured resin adhesives with and without EgMA (mean and SD; n=4). *Differences were statistically significant with respect to the corresponding unmodified bonding agent (*P*<0.05)

Fig. 5.1-6 shows representative 3D volume reconstructions of biofilm sections by using *bio*Image_L (Chávez de Paz 2009). The percent cell viability measurements (green biovolumes) were high for CUB (>77%) and SBMP (>90%) control adhesives compared with their corresponding modified adhesives that showed more dead (red biovolumes) cells.

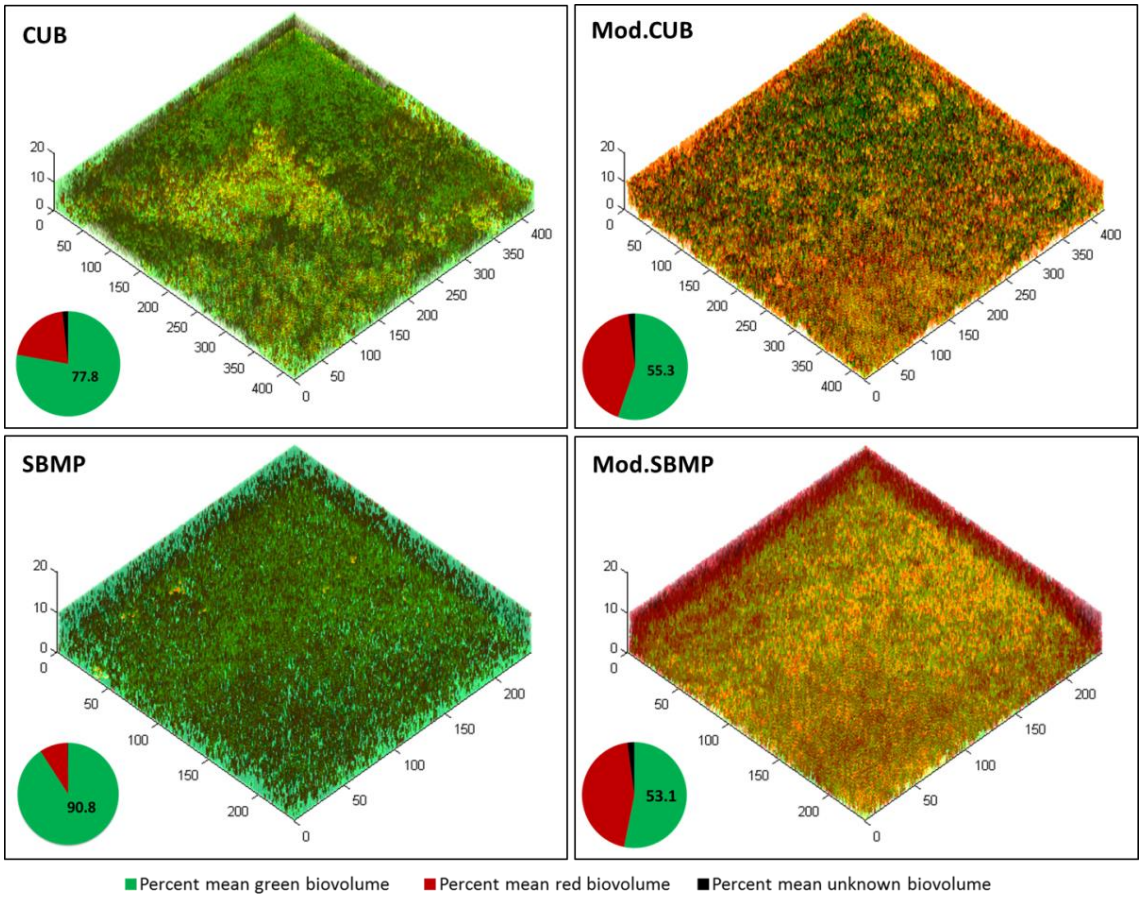

Figure 5.1-6 BioImage L analysis results: Representative 3-D images of 14 days microbial biofilms grown on cured resin discs of the four adhesives tested with live/dead staining. The pie charts show the effect of EgMA incorporation on the mean percentages of dead (red), live (green) and unknown (black) biovolumes

5.1.3.7 Cytotoxicity

All adhesives investigated in this study showed acceptable biocompatibility at 24 h and 48 h exposure time showing a high percentage of cell viability (Fig. 5.1-7), above 70% of the negative non-toxic control (the minimum cell viability percentage below which the material has a cytotoxic potential, ISO 10993-5).

The statistical analysis revealed no significant reduction in cell viability of EgMA modified adhesives comparing to their unmodified native adhesives (P<0.05) at 24 h and 72 h elution times. The results also showed that among all groups, the 72 h extract of CUB group only exhibited significantly higher cytotoxicity comparing to the negative control at both exposure times. For all groups, no significant differences were found between 24 h and 48 h exposure time.

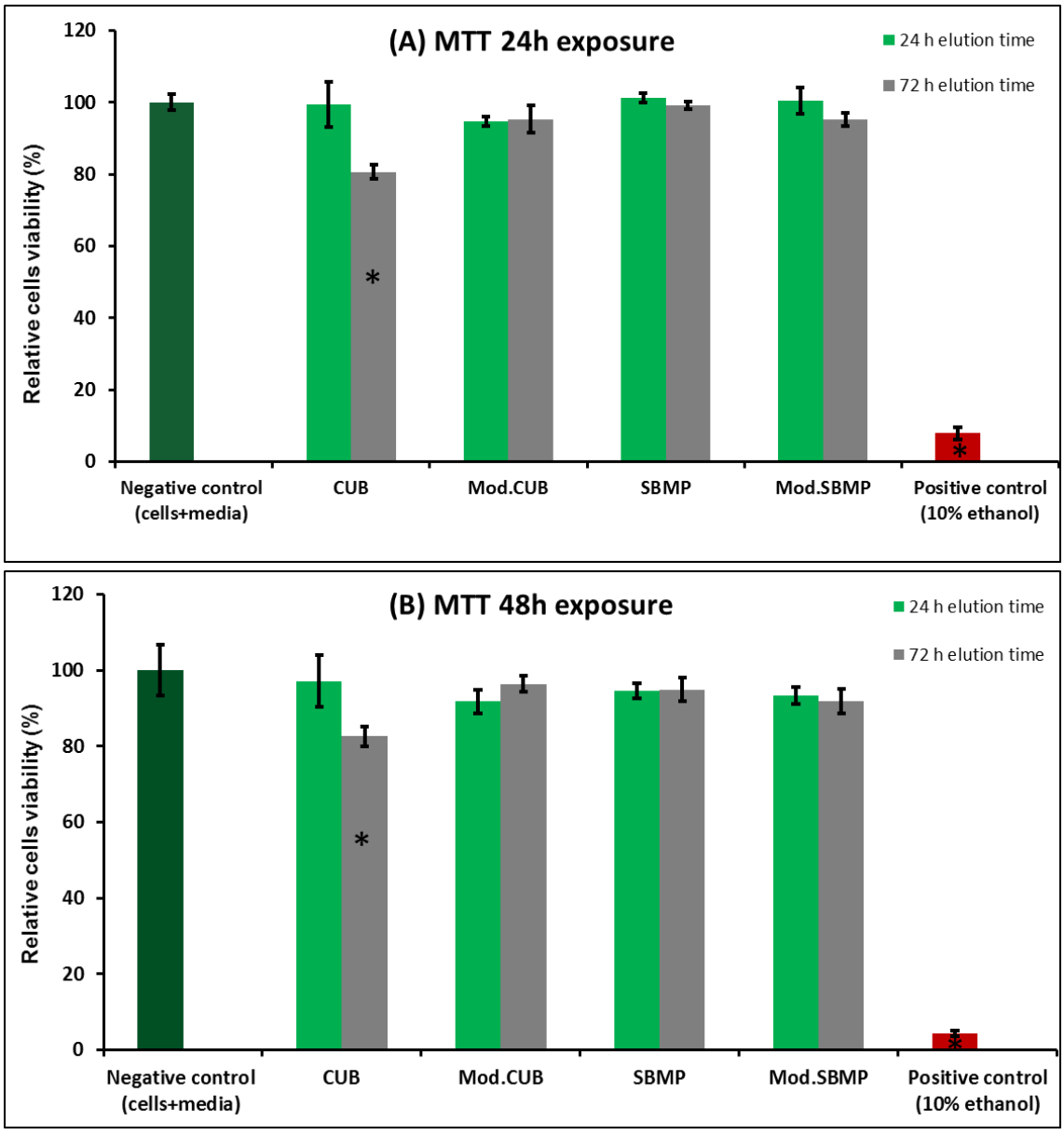

Figure 5.1-7 Viability of human gingival fibroblast (HGF) cells following exposure for (A) 24 h and (B) 48h detected at 24-h and 72-h eluted media from EgMA modified and unmodified adhesives tested. The relative cell viability is presented as a percentage of the negative non-toxic control group (n=5). *Denotes significant difference when compared with negative control (P<0.05)

5.1.4 Discussion

5.1.4.1 Curing and thermal properties

The curing ability of a bonding system is considered to be one of the important factors for obtaining a strong and durable bond to the dentinal substrate. The degree of conversion (DC) observed 10 min post curing (Table 5.1-2) of modified bonding agents containing 20% EgMA was significantly lower, which increased when measured at 24 h with values comparable to formulations without EgMA, indicating post-curing. The initial reduction in the degree of conversion is the consequence of the bi-functional nature of acrylic and allylic double bonds in the EgMA moiety, which further confirms the participation of the monomer during the bulk polymerisation (Rojo *et al.* 2006) leading to either branching or crosslinked structures with unreacted allylic bonds from the pendant eugenyl moiety. These results indicate that addition of EgMA had no adverse effect on curing behaviour of Bis-GMA/HEMA-based bonding resin similar to the results reported previously for Bis-GMA/TEGDMA based resin systems for composites (Almaroof *et al.* 2016a).

However, the DC does not give a complete characterisation of polymer structures, for this reason, the *T*g of each adhesive was also measured. *T*g may reflect the extent of crosslinking by which the mechanical and physical properties of a polymer are determined. Here, despite the lower *T*g of EgMA homopolymer (about 95 °C) (Rojo *et al.* 2008a) in comparison with Bis-GMA, the main methacrylate derivative within the formulations of both adhesives tested, the modification with EgMA monomer resulted in similar *T*g values (Fig. 5.1-1). This confirms the formation of slight crosslinking or branching within the network structures which potentially increased the physical properties and stability of these bonding agents (Tamareselvy and Rueggeberg 1994). This finding was also consistent with previous studies in which EgMA was copolymerised with other methacrylate monomers, ethyl methacrylate (EMA) (Rojo *et al.* 2008b) and 2-hydroxyethyl methacrylate (HEMA)(Rojo *et al.* 2008a).

Furthermore, the presence of acrylic and allylic double bonds in the EgMA moiety could also lower the rate of reaction resulting in lower peak temperatures. The reduction in the exothermic polymerisation (about 12 °C) was significant for both Mod.CUB and Mod.SBMP adhesives, which exhibited peak temperatures of 54.0 °C and 86.6 °C respectively. This constitutes an additional advantage preventing thermal damage to adjacent root dentine and periodontal ligaments whilst the polymerisation occurs within the root canal system.

5.1.4.2 Water sorption, solubility and wettability

The physical and mechanical properties of adhesive polymers may be significantly altered by the effects of water uptake and elution of components, which results in polymer swelling, plasticisation and catastrophic degradation of resin-dentine bonds (Hosaka *et al.* 2010). Fluid uptake in an oral environment could lead also to bacterial harbouring and marginal discolouration.

The chemical composition of adhesive resins and their net hydrophilicity have an effect on water sorption, solubility and water diffusion in these polymers. The presence of acidic, highly polar functional groups increases the diffusion of water molecules through the polymer matrices by binding successively to the polar sites via hydrogen bonding. Another factor that can affect the extent and rate of water uptake is the cross-link density of these polymer networks, which decreases the hole-free volume for water diffusion and subsequently reduces the water permeability of the polymer. The hydrophobic nature of EgMA substituted on methacrylates and its ability to form slightly cross-linked structures significantly increased the hydrophobicity of both modified adhesives and resulted in a significant reduction in their water sorption values. This finding was also in agreement with a previous study in which high conversion copolymers from the hydrophilic monomer HEMA and EgMA were prepared demonstrating lower sorption with increased eugenyl moieties (Rojo *et al.* 2008a).

The water sorption is usually associated with a solubility, which is measured as a loss of dry mass in samples that have been immersed in water over time. The residual unreacted monomers or oligomers may be released from swollen dental adhesives during water sorption and subsequent polymer relaxation. Here, the self-etch adhesives showed higher values of water sorption and solubility than that of total-etch adhesives (Table 5.1-3). This is probably associated with the presence of the phosphate group and residual solvent in their composition that is in agreement with the results of a water sorption study on both solvated and non-solvated commercial adhesives (Malacarne *et al.* 2006). It has been reported that residual solvents increase the free volume of polymers and can promote water sorption even after its evaporation prior to water immersion (Hosaka *et al.* 2010). Furthermore, using the data obtained from SFE and contact angles, it is obvious that these adhesives also presented a more hydrophilic behaviour than total-etch adhesives. More hydrophilic polymer networks permit a rapid release of unreacted monomers through nano-voids in the material because of the higher relaxation capacity (Brazel and Peppas 1999), showing a decrease in weight within a short time of water immersion (Fig. 5.1-2). Therefore, the results of the present study indicate that the incorporation of EgMA into the self-etch adhesive formulation may be clinically useful. The modified CUB one step adhesive showed a significant reduction in solubility, limiting the extraction of any unreacted components into the surrounding environment potentially resulting in increased mechanical stability and long-term durability of resin-dentine bonds.

The wettability of the adhesive polymers was studied because of its importance in the material-medium interactions. Thus, the surface properties were evaluated by measuring the contact angle and SFE as parameters of surface hydrophobicity considering two liquids of opposite polarity, water and methylene iodine. However, the effect of SFE of substrates on bacterial adhesion has been critically discussed in the literature with no clear consensus. Bacterial adhesion is a complex phenomenon and is related to the surface energy of substrate and bacteria. It has been reported that the total interaction

energy between the bacteria and the substrate linearly increases with the decrease of the surface energy (Absolom *et al.* 1983; Liu and Zhao 2005). Taking into consideration the previous findings for experimental polymers and composite incorporating EgMA, the greater hydrophobic character of these materials, presented a lower SFE (Almaroof *et al.* 2016b), improved the total interaction energy with the bacteria and resulted in a higher accessibility to the eugenyl moieties responsible for the bacteriostatic activity (Rojo *et al.* 2008a).

5.1.4.3 Push-out bond strength and interface evaluations

The results of the current study showed that irrespective of the adhesive system, the incorporation of EgMA did not adversely affect the root dentine bonding ability of the parent adhesives. The adhesive systems selected in this study were based on two different bonding strategies, self-etch and total-etch adhesive systems. They differ in composition and are usually used in association with resin cement or composites core materials to bond the endodontic restoration to root canal dentine. EgMA was successfully incorporated into the commercial adhesives with no evidence of phase separation or agglomeration. This was confirmed by the results obtained from curing and thermal characterisation discussed above and reflected the absence of any adverse effects on their bonding characteristics. The ability of these adhesives to bond a resin composite to the coronal and middle regions of the root canals was evaluated by push-out test, which has been accepted as a reliable method for measuring the bond strength to root canal dentine (Goracci *et al.* 2004). In order to test solely the composite-dentine interface in an optimum standardised way, while excluding the composite-post interface, no posts were inserted into the cavities. The push-out bond strength values obtained from all adhesives tested were within the range of previous studies on root dentine adhesion using different dental adhesives with resin composite materials (Lee *et al.* 2014; Zavattini *et al.* 2014). Although the push-out strength of the self-etch adhesive CUB was not significantly higher with the inclusion of EgMA, the regional bond strengths,

revealed that it created a significantly higher (*t*-test; $P<0.05$) bond strength in the middle region of the canal.

CUB is a single-step adhesive which combines the primer and adhesive into one bottle, contains a strongly acidic adhesive monomer, MDP enabling simultaneous demineralisation and monomer penetration into the dentine and microfiller. Since the addition of EgMA does not affect the pH value of the adhesive (data not shown), it is speculated that the same degree of demineralization of the smear layer as the control CUB is obtained for the Mod.CUB. The improvement in bond strength could be attributed to the reduction in viscosity observed after the addition of the monomer, which might enhance the infiltration of the adhesive resin into the root dentine. The low viscosity of the EgMA monomer functioned as an excellent diluent for Bis-GMA within its composition, which lowers the initial viscosity of the monomer mixture.

SBMP is an etch-and-rinse adhesive that requires surface etching and priming before its application. The EgMA modified components of this adhesive system showed chemically comparable compatibility with its water-based primer after their application into the acid etch dentine surface and air-drying, exhibited no significant differences in push-out bond strength value in comparison to its control.

Failure mode analysis showed adhesive failure between composite and dentine and mixed failure pattern were the most common failure types for both adhesives in control and modified groups (Table 5.1-4). However, the modified CUB demonstrated higher mixed and cohesive failure indicating the superior quality of the bond. In general, bond strength to root canal dentine is lower than bond strength to coronal dentine (Mannocci *et al.* 2001a). Several studies have reported adhesive failures predominantly between post space dentine and resin cement or composite materials (Foxton *et al.* 2005; Bitter *et al.* 2009b). Furthermore, the SEM indicated similar interfacial morphological features for control and modified antibacterial adhesive systems. The de-bonded specimens often presented a residual resin covering a well-hybridized dentine surface in the coronal and

middle regions of the roots. The results of SEM examination were in agreement with the findings of confocal microscopy images, showing a gap-free continuous resin-dentine interface with a clear hybrid layer in both coronal and middle regions of the roots, indicating the ability of Mod.CUB and Mod.SBMP to diffuse into self-etch and acid-etch root dentine respectively. The mix between adhesive components and primer components is also clearly observed through images shown in Fig. 5.1-4C and D. Thus, it is possible to conclude from the SEM and CLSM evaluation that the incorporation of EgMA does not impair the bonding ability of the parent adhesives applied on to root canal dentine.

5.1.4.4 Antibacterial activity

The main cause of refractory endodontic infection is the persistence of bacteria in the root canal space (Siqueira 2001). Cavity disinfectant, such as chlorhexidine are often used by dental clinicians before application of bonding-systems as complete removal of bacteria from the root canal system is not achievable after careful cleaning and shaping (Oguntebi 1994). However, chlorhexidine is immiscible with dental monomers and does not undergo any polymerisation. The dissolution of chlorhexidine aggregates leads to the formation of a porous surface, which potentiates staining, bacterial biofilm accumulation and leading to detrimental mechanical properties of the polymers (Imazato 2003). On the other hand, if adhesive systems possessed inherent antimicrobial activity it can in addition function as a cavity disinfectant agent before polymerisation which reduces clinical chair time, furthermore, the cured bonding will inhibit or reduce the growth of oral and root canal bacteria on its surface by the immobilised agent.

The ADT as standard assay was used initially in this study to determine the potential antibacterial effectiveness of unpolymerised adhesive resins containing EgMA monomer against three oral bacteria. The bacterial species used are usually prevalent in primary or secondary root canal infections (Pinheiro *et al.* 2003). *S. mutans*, virulence factor include its ability to synthesise adhesive glucans and generate acids that result in the demineralisation of dental tissues, thereby initiating dental caries on tooth structure

(Tanzer *et al.* 2001). *E. faecalis* can remain viable even after chemo-mechanical preparation (Oliveira *et al.* 2007) constituting a source of refractory endodontic infections and *P*. acnes is an opportunistic pathogen responsible for a wide range of infections and inflammatory conditions (Niazi *et al.* 2010; Niazi *et al.* 2016).

Both Mod.CUB and Mod.SBMP produced significantly greater inhibition zones than their corresponding controls indicating that the eugenyl residue in EgMA monomer maintains the bactericide effect of eugenol and the sensitivity of the three bacteria to this monomer within their formulations. Among the selected bacteria, EgMA containing adhesives produced significantly greater inhibition zones against *P. acnes* than *S. mutans* and *E. faecalis* (P<0.05). These findings are in general agreement with previously reported studies on the eugenol and eugenol containing materials effect on *E. faecalis* (Dorman and Deans 2000), *S. mutans* (Kaplan *et al.* 1999; Rojo *et al.* 2006; Xu *et al.* 2013) and *P. acnes* (Viyoch *et al.* 2006).

It is important to emphasise that some inhibition zones were also observed with unmodified control adhesives, especially with CUB. This is in agreement with previous reports and could be attributed to the presence of components that are originally incorporated to promote adhesion (Stephanatac *et al.* 2001) and to the lower pH environment of self-etch adhesives containing more acidic monomers MDP (Ohmori *et al.* 1999). However, it was acknowledged that the low pH exhibited by conventional dental bonding agents is not sufficient to ensure a reliable bactericidal activity because the acidity of these adhesive can be neutralised by the buffering action of the medium (Imazato *et al.* 2006). In the present study, both control adhesives tested showed no inhibition against *S. mutans* which might able to survive acidic pH (Li *et al.* 2002). It is, therefore, expected that the new formulations have the potential to kill residual bacteria in the root canal space.

A multispecies *in vitro* biofilm model was successfully established on the surface of cured adhesive resin discs to evaluate their inhibition effectiveness against biofilm formation.

In our previous study, the antibacterial activity of EgMA containing composites against the adherence and growth of individual species was reported (Almaroof *et al.* 2016b).

Oral bacteria *in vivo* colonise on a substrate surface to form biofilms and display properties that are dramatically distinct from their planktonic analogue in term of antimicrobial agent resistance, which makes their elimination much more difficult. The biofilm model used in this study was nutritionally stressed to mimic the environmental conditions of the root canal system following the protocol of nutrient-stressed biofilm (Niazi *et al.* 2014). The previous study showed that after the initial 7 days of regular medium replenishment, the fermentation of glucose results in the production of acid, lowering the pH of broth supernatant. Leaving the biofilms for 7 days in the same medium led to the complete consumption of glucose from broth supernatant; thus, leaving the biofilm bacteria starved of the depleted fermentable carbohydrates resulting in the degradation of serum proteins and release of ammonia causing an eventual rise in the pH. The biofilm communities of refractory endodontic infections are surviving the inaccessible nutrients and pH change that possibly render them resistant to the intracanal procedures of disinfection (Siqueira 2002).

The inclusion of 20 wt.% EgMA achieved a stronger effect in reducing the biofilm viability, evidenced by decreases in the number of CFUs of the total species, live biofilm volume and percentage of live bacteria (Fig. 5.1-6). The antibacterial activities of the essential oils including eugenol have been assessed and reported (Dorman and Deans 2000; Chang *et al.* 2001). Although the mechanisms by which these compounds exert their activity are not fully understood, it is well-known that Gram-positive bacteria are more sensitive to the essential oils, as the hydrophilic cell wall structures of Gram-negative bacteria block the penetration of hydrophobic components in the cell membrane (Burt 2004). In the case of EgMA monomer, the data suggest that the presence of a conjugated double bond in its aromatic structure and the allylic side chain is behind the strong inhibitory effects of this component (Rojo *et al.* 2008a). In addition, its activity may be further explained in terms of the alkyl substitution into the phenol nucleus. This alters the

distribution ratio between the aqueous and the nonaqueous bacterial phases by reducing the surface tension (hydrophobicity of the compounds) or altering the species selectivity (Dorman and Deans 2000).

Thus, the modification of the chemical structure of the eugenol molecule to obtain the α-unsaturated ester, EgMA results in a good hydrophobic balance with a certain proton exchange capacity. This together with the presence of the 4-allyl group allows the monomer to maintain the ability to alter the cytoplasmic membrane permeability with a consequent block of ionic pumps (Rojo *et al.* 2008a). The data also indicate that the disruptive effect on cytoplasmic membranes is maintained after the polymerisation reaction indicating that EgMA based materials render bioactive bacteriostatic surfaces that reduce microbial resistance and biofilm formation. The respective virulence of each species and the differences in the chemical composition and structure of the bacteria cell walls resulted in different bacterial sensitivities toward EgMA. From ADT results, *E. faecalis* is the most resistant bacterium to EgMA monomer and *P. acnes* is the least (Table 5.1-5). Further study is needed to identify their individual colony morphology on selective media with biofilm inhibition test. However, the effectiveness of EgMA modified adhesives against *E*. *faecalis*, which is one of the resistant bacteria to a wide range of antibiotics, offers a considerable advantage over the commercially available dental adhesives.

5.1.4.5 Cytotoxicity

Antibacterial agents whilst inhibiting bacterial growth should also be minimally cytotoxic for mammalian cells. As is known, eugenol at high concentrations can exert some toxic effects on the dental pulp (Markowitz *et al.* 1992). Eugenol and related compounds were shown to have a high affinity for plasma membranes because of their lipid solubility, which could contribute to cell damage (Manabe *et al.* 1987). However, the cytotoxicity of EgMA monomer and methacrylate polymer matrices containing EgMA with human fibroblast has been reported earlier to show good cytocompatibility (Rojo *et al.* 2006).

The cytocompatibility of EgMA modified adhesives exhibited HGF viability matching that of a commercial non-antibacterial control, and that of the negative control without any resin eluent. Interestingly, the Clearfil Universal Bond™ extract at 72 h exhibited significantly higher cytotoxicity compared to that at 24 h elution time and to all other groups. The acidity of the extract, which contains the acidic monomer MDP in the self-etch adhesive, may lower the pH, thus reducing cell viability significantly. However, this difference was not found in Mod.CUB group suggesting that the ability of the monomer to form a slightly cross-linked network, which limited the amount of possible leaching of the acidic monomer.

5.1.5 Conclusions

The EgMA modified self-etch and total etch dental adhesives showed antibacterial activity before and after curing against a range of endodontically pathogenic bacteria and produced an effective bond to root canal dentine and high compatibility *in vitro*, indicating a potential application to achieve successful post-endodontic restorations.

5.2. Supplementary information on durability of EgMA modified adhesives

Thirty-six human single-rooted premolars were used to evaluate the bonding ability of adhesives after ageing. The experimental details on adhesive application, root sectioning, push-out bond strength and CLSM specimens labelling are described in sections 5.1.2.8 and 9. The ageing of the root segments was carried out by storing in distilled water at 37 °C for six months before sectioning and testing. Statistical analysis was conducted and the influence of EgMA incorporation on total and regional bond strengths of each material, at 6 M ageing, was investigated using *t*-tests. The push-out bond strengths and the percentages of the failure modes are shown in Table 5.2-1. The total and regional bond strengths of both modified adhesives were statistically non-significant ($P>0.05$) compared to the controls. The failure analysis was in accordance with the push-out bond strength results; all groups failed mainly in adhesive mode. The Mod.SBMP adhesive showed the highest total bond strength value among the groups with more mixed and cohesive failures.

Table 5.2-1 Comparison of the mean push-out bond strength (MPa) values and the analysis of failure modes of the experimental adhesives tested after 6 M ageing

Bonding agents	Bond strength values (mean [SD])			Total failure mode (%)		
	Regional BS (n=12)		Total BS (n=24)	Adhesive	Mixed	Cohesive
	Coronal	Middle				
CUB	8.2 [2.9]	4.7 [1.8]	6.4 [2.9]	79	21	0
Mod.CUB	9.1 [3.3]	6.5[2.3]	7.8 [3.1]	67	29	4
SBMP	9.5[3.3]	5.8 [2.6]	7.6 [3.5]	67	29	4
Mod.SBMP	12.2 [3.7]	6.1 [2.8]	9.1 [4.5]	54	38	8

The comparisons with the immediate bond strengths (24 h storage) are shown in Fig. 5.2-1. A linear regression model was used to test the effect of materials, regions and time on bond strength. The results showed that there was no interaction between materials & region and region & time (*P*=0.13 and 0.17, respectively). However, the interaction between material and time was statistically significant (*P*=0.03) and hence further post-hoc analyses were carried out to find out which combination was significant. The total bond strengths of six-months-aged samples of CUB, Mod.CUB and SBMP adhesives were significantly decreased, whilst the bond strength of Mod.SBMP was not significantly altered after ageing. Adhesive failure mode significantly increased in all groups over the storage period (Table 5.2-1).

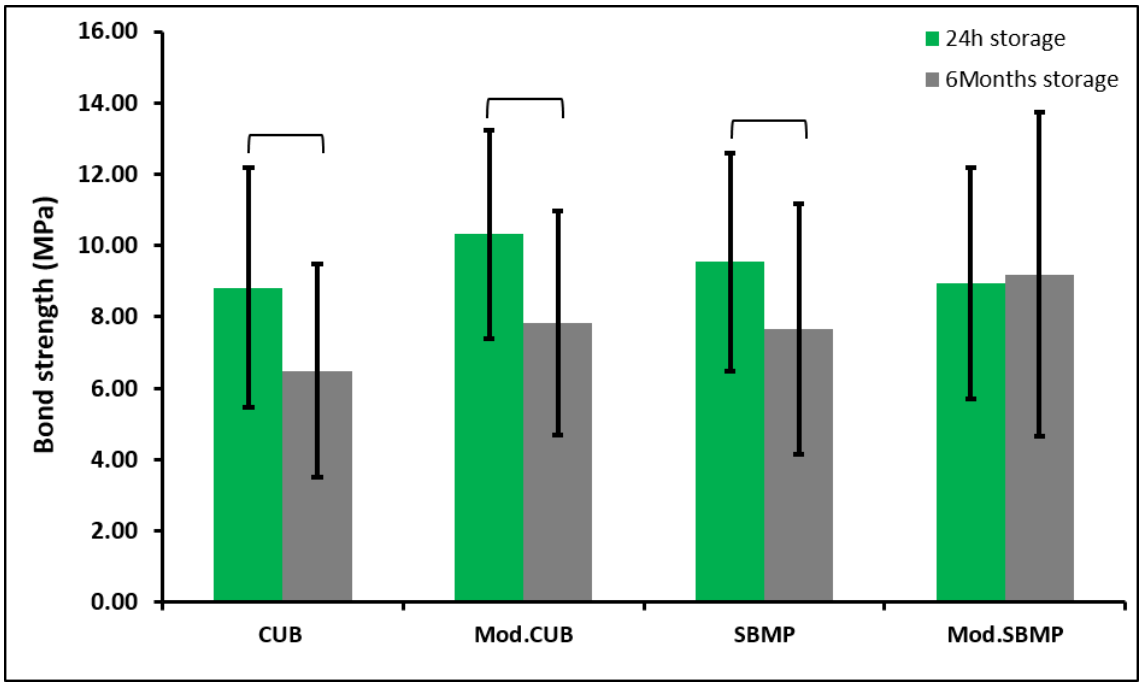

Figure 5.2-1 Comparison of push-out bond strengths between immediate and aged adhesives groups tested in this study. The connection with horizontal lines indicates significant differences between groups after 6 M ageing (*P*<0.05)

Water storage is the most common artificial ageing model for long term resin-dentine bond evaluation (Oyagüe *et al.* 2009). It can simulate the moist intraoral environment when the restored teeth are continuously surrounded by saliva. Literature reports show that the bond degradation occurring during ageing is a complex process, involving the hydrolysis of both the resin and the collagen fibril phases contained within the hybrid

layer (Breschi *et al.* 2008; Frassetto *et al.* 2016). It has been thought that the host-derived proteases with collagenolytic activity (MMPs) take part in this phenomenon (Pashley *et al.* 2004). However, hydrolysis is considered a primary cause for resin degradation within the hybrid layer, contributing to the reduction of bond strength over time.

Water sorption is an unavoidable process; the prolonged exposure to oral fluids promotes the chemical hydrolysis of ester bonds in methacrylate materials (Ferracane 2006). The subsequent hydrolysis and leaching of the hybrid layer components, which correlates with adhesive hydrophilicity and water uptake (Tanaka *et al.* 1999; Ito *et al.* 2005), weaken the bond and lead to failure of the adhesive interface with clinical debonding. The failure is usually combined with microgaps formation that allows the penetration of pathogens. Therefore, the presence of bacteria, esterases, and dental biofilm (Bourbia *et al.* 2013) induces a cascade of events resulting in bond interface degradation and failure of the restoration.

Hence, the addition of EgMA antibacterial agent could offer a considerable benefit over the contemporary dental adhesives by inhibiting the bacterial growth and thereby preventing the deterioration of the adhesive interface. In addition to that, the ability of the monomer to enhance the hydrophobicity and reduce water uptake of the modified adhesives (Almaroof *et al.* 2016b) could also play a role in bond interface ageing and stability; however, this effect was more evident with the three-step adhesive. The Mod.SBMP total-etch adhesive effectively displayed higher bond stability than Mod.CUB, since no statistically significant differences were found between immediate and aged specimens.

The durability of resin-dentine bonds was found to be vary between different adhesive systems. According to a recent systematic review, the water storage of self-etch adhesives showed lower bond strength when compared to etch and rinse adhesives (Masarwa *et al.* 2016). The results obtained in this study after EgMA modification are in agreement with previous studies, suggesting that the simplified one-step adhesives and

self-etching primers are more susceptible to hydrolytic degradation due to the intrinsic hydrophilicity by virtue of the acidic monomers and methacrylates with polar substituents (Tay *et al.* 2002; Abdalla and Feilzer 2008; Feitosa *et al.* 2014). Thus they are able to rapidly absorb water, which results in polymer swelling, plasticisation and weakening of the polymer network (Ito *et al.* 2005). The post-curing polymerisation that extends beyond the 24 h storage, increasing the conversion degree of the adhesive resin, could also contribute to better bond strength after ageing.

The bond strength data were further supplemented by SEM findings that revealed the dentine side of aged specimens, which failed in adhesive mode or fractured through the hybrid layer (Fig.5.2-2). In general, increased adhesive resin (r) loss and exposed dentinal tubules (t) were observed in specimens stored for 6 M, which were more evident in resin-dentine interfaces prepared with self-etch adhesive (A&B), indicating the degradation of the hybrid layer over time. Porous resin tags are also evident (arrow). These findings are consistent with previous observations on resin-dentine interfaces ageing (Hashimoto *et al.* 2003; Yesilyurt *et al.* 2015). Representative images of Mod.SBMP (D) and SBMP (C) adhesives show an important presence of adhesive resin with a well-formed hybrid layer on dentine surface, and it is possible to observe that most of the dentinal tubules were obliterated with resin tags (rt) with few exposed tubules (t), especially in the coronal region of the canal.

The confocal microscopy evaluation performed after 6 M ageing is shown in Fig. 5.2-3. It is possible to observe a uniform hybrid layer and resin tags located under the adhesive layer for both modified and control groups. Gaps were also observed between the hybrid and adhesive layers, especially with CUB self-etch adhesives Fig. 5.2-3 A2 and B2. However, an important potential limitation of CLSM imaging of labelled adhesive interfaces after moisture ageing is the possibility of dye partition from the labelled material because of its water solubility (Watson 1997).

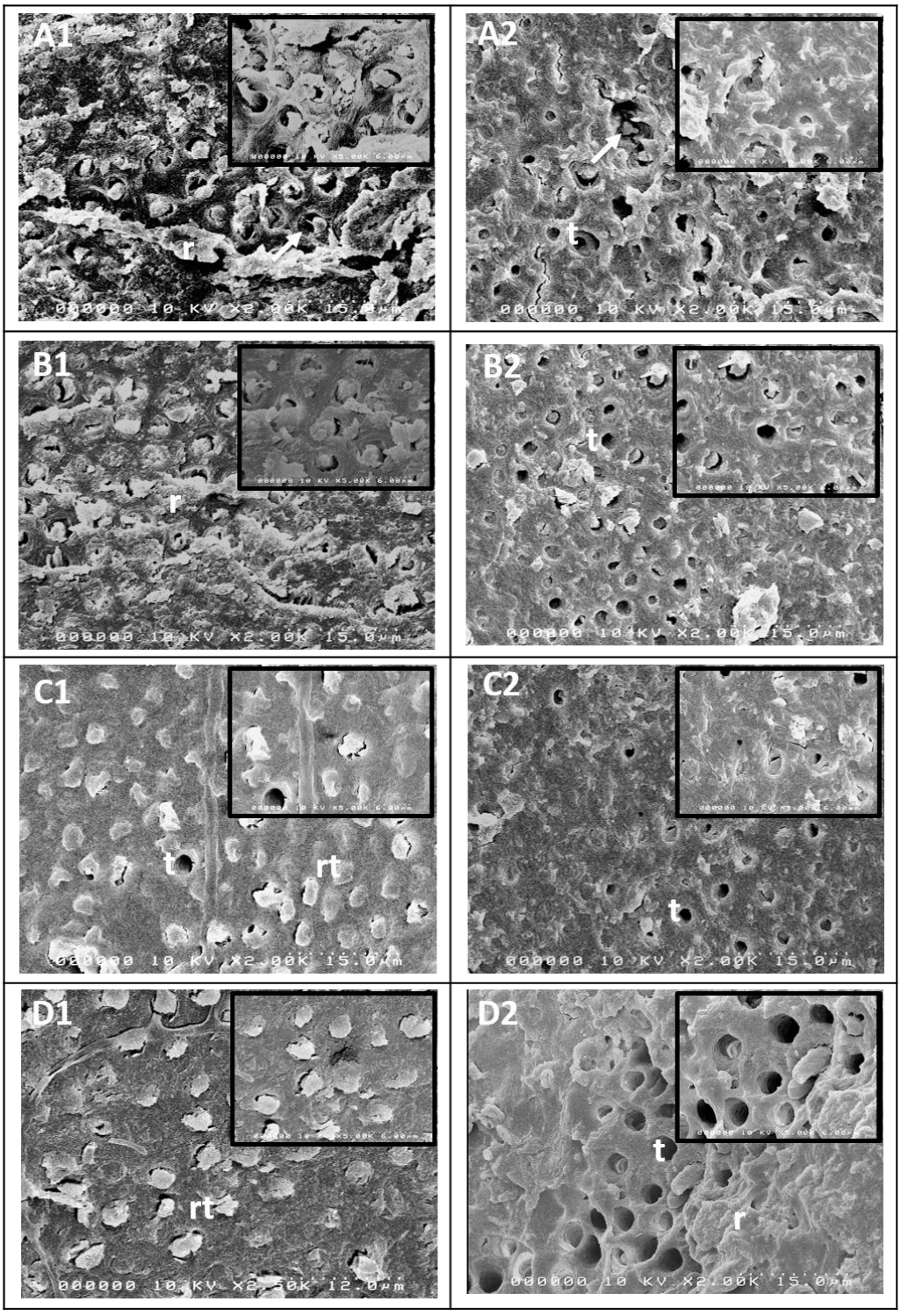

Figure 5.2-2 SEM images of debonded specimens created using the modified adhesives (A:CUB, B:Mod.CUB) tested in comparison with their control (C:SBMP, D:Mod.SBMP), taken from coronal (1) and middle (2) regions after 6 M of aging in distilled water at 2000x and 5000x magnifications showing the features of failure in the adhesive layer. r, resin; t, dentinal tubules; rt, resin tags.

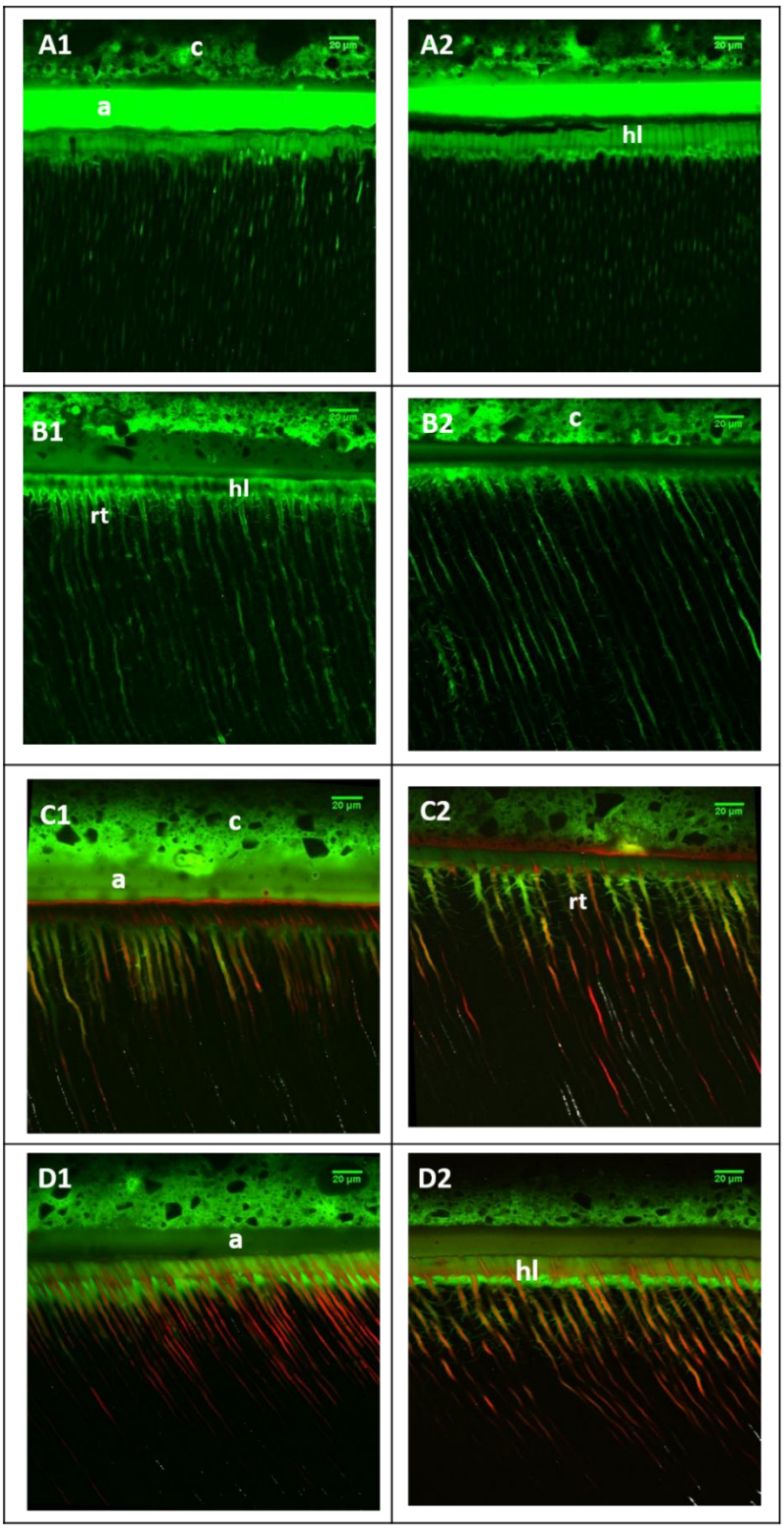

Figure 5.2-3 Confocal laser scanning microscopy (CLSM) images showing the interface of the resin-dentine created using the four adhesives tested (A: CUB, B: Mod.CUB, C: SBMP, and D:Mod.SBMP), from coronal (1) and middle (2) regions of the root after 6 M storage in distilled water. a, adhesive layer; c, composite; hl, hybrid layer; rt, resin tags.

Chapter 6 General discussion, conclusions & suggestions for future work

6.1 General discussion

The main objective of endodontic therapy is to eliminate bacteria and provide a treatment that allows restoration of a tooth that is damaged due to trauma, pulpal and or periapical diseases. Nearly 15 million people are treated annually via non-surgical root canal treatments to retain function and avoid extraction of the tooth (ADS 2007). Although the success rate of RCT is high, the complex anatomy of the root canal system in conjunction with the presence of resilient pathogenic bacteria, limitations of current chemo-mechanical instrumentation and obturation methods often limit the success of the procedure. Not all endodontically treated teeth require a post and core restoration, but it is usually indicated when there is a need to replace the missing coronal tooth structure and provide retention and resistance for the overlying restoration (the crown). The prognosis of the endodontically treated teeth also drops with extensive loss in tooth structure (Bitter *et al.* 2009a; Signore *et al.* 2011). The loss of such teeth can lead to loss of oral function and appearance which impacts ultimately on function, output and quality of life. This presents the dental practitioner with the challenges of predicting the survival of ETT and complicates the clinical decision as to whether such teeth be retained or extracted.

The main reason for extraction of ETT is not failure of endodontic treatment, but caries, inadequate restoration, and root fractures (Baba *et al.* 2017), hence the quality of coronal restorations is crucial for the final outcome; bacterial growth or contaminations must be prevented. These considerations are the most critical components in the success of post-core restorations, which have led to a number of new restorative materials, with some being launched commercially in the last decade (Seemann *et al.* 2014).

The review of the relevant background literature identified that the restoration of structurally compromised ETT with adhesively luted fibre posts and direct resin

composites is a treatment of choice, which conserves remaining tooth structure and provides the most reliable results (Grandini *et al.* 2005b; Juloski *et al.* 2014; Murali Mohan *et al.* 2015). Although laboratory studies showed the favourable mechanical and physical properties of fibre posts, clinically, a wide range of failure mechanisms have been documented in the literature (Table 1.3), indicating the limitations of currently utilised materials. Thus, the overall aim of this research was to develop, characterise, and assess newer restorative materials for effective restorations of ETT.

New composite post materials based on a thermoplastic polymer matrix, LDPE reinforced with hydroxyapatite and zirconium oxide was developed using a melt extrusion process, designed to specifically overcome the rigidity of the metallic and FRC posts. The composites were designed to closely mimic an intact tooth with respect to mechanics, aesthetics and with a strong focus on retrievability of the material, in case of a need to retreat the tooth, which is often not given proper consideration.

Polyethylene has generated a great deal of interest for biomedical and dental applications because of its excellent biocompatibility, thermoplasticity, processability, and high flexibility (Whitehouse *et al.* 1999). The low coefficient of friction and the modulus of elasticity being close to bone prompted the application of ultrahigh molecular weight polyethylene (UHMWPE) in fabricating components of joint replacement prosthesis. Polymer composites with hydroxyapatite as the filler were first introduced by Bonfield *et al.* (1981) and the effect of HA on the mechanical properties of the composite HDPE/HA were reported in order to be applied for bone replacement. The study clearly showed that Young's modulus improved with the inclusion of hydroxyapatite, yielding a biocompatible composite for potential biomedical applications. Novel hydroxyapatite based dental composites have been reported with improved mechanical properties (Labella *et al.* 1994) and more recently HA whiskers in resin composites showed comparable mechanical properties with 20% filler and much improved cytocompatiblity (Calabrese *et al.* 2016).

The ready processability by all thermoplastic methods and the chemical resistance of LDPE led to the fabrication of the composites using single screw extruder, a simple polymer processing technique, where a thermoplastic material is forced through a die orifice to produce continuous length composite fibres with a uniform cross-section. This technique provides intensive dispersive mixing of fillers and additives with excellent temperature control. Hence, to adapt this technology for developing a post material, a pilot study was undertaken to identify the processing variables and obtain materials, compliant with the requirements of post-core materials. The amount of filler is a significant parameter that helps in tailoring composites with the desired degree of flexibility, especially in view of the requirements for the clinical placement of posts. The pilot studies showed that 50% of HA yielded a brittle fibre, hence lower concentrations of 30 and 40% by weight of HA were used in the main study. The effect of silanation of the HA particles was also established from this study (section 2.2.1) that clearly indicated that silanation was necessary to enhance the interfacial adhesion between filler and matrix.

Accordingly, two sets of composite fibres were produced by incorporating 30 and 40% by weight of silanated HA particles into the low-density polyethylene resin matrix with 20% and 10% ZrO_2, respectively. The mechanical properties of the composites improved as expected and were in agreement with previous findings of LDPE/UHMWPE–HA composites (Tanner 2010; Alhashimi *et al.* 2013). The increasing amount of zirconia imparted the desired level of radiopacity without compromising the physical and thermo-mechanical properties of the composites. The silanation of the HA particles enhanced the bond interfacial adhesion between filler and matrix, which contributed to low water uptake and maintained the flexural properties over three months' storage in SBF. SEM examination showed how PE shrink around individual particles during thermal processing to form a homogenous mixture indicating better compatibility and durability of the composite material (Fig. 2.1-5).

The rationale for introducing the thermoplastic polymer in post fabrication was to create a less rigid biomimetic material. In the past, clinicians and researchers have assumed that a post should be rigid (Torbjörner *et al.* 1996), but rigid posts transfer stresses to the less rigid component, the root dentine, which may result in mechanical failure. The structural flexibility of PE-HA composites suggests their potential application to restore flared or wide root canals. Large flared canals, which result from caries, anomalies, internal resorption, and over-preparation (Latempa *et al.* 2015), are more susceptible to fracture because of the thin walls remaining, thereby requiring biomechanical support by the restorative material that does not compromise the integrity of the remaining radicular tooth structure (Flanagan 2014). However, because the mechanical properties of posts depend largely on the diameter (Lambjerg-Hansen and Asmussen 1997), the lower flexural modulus (3.0 to 3.3 GPa) and flexural strength (54 to 64 MPa) of PE-HA composites may restrict their potential application in a small diameter post when compared to currently used glass and quartz FRC posts.

The LDPE-HA posts had the distinct advantage of being easily removable in case of retreatment. The thermoplastic LDPE with a low melting temperature of 135 °C enabled the removal by using existing conventional dental devices. The removal procedure of PE-HA post was proposed in a special protocol, and a pilot study to evaluate the removal time was carried out *in vitro* (section 2.2.2). This procedure was found to be extremely rapid and safe in comparison with the other techniques. The results showed that the experimental PE-HA post required less removal time (88 ± 23.8 s) than the currently used fibre posts (literature data, Table 2.2-3). Since highly cross-linked thermoset polymer matrices are used in manufacturing fibre posts (usually epoxy resin), it complicates removal. Most current techniques involve enlarging the channel around the residual post or progressive drilling through the middle of the post, which is associated with traumatic loss of remaining root canal dentine. Although the LDPE-HA/ZrO_2 composites have desirable properties for fabrication of posts, the mechanical properties

need further refinement; however, this study indeed provides a new approach for endodontic post material development in the future.

The current trends in minimally invasive dentistry and aesthetic demands have seen the advent of post and core systems that are replacing the conventional metallic posts. The development of methacrylate monomer, bisphenol-A-glycidyl-methacrylate (Bis-GMA) and dental composites has been extremely successful as aesthetic restorative materials, which have been modified through reduction in size of the filler particles and the use of fibre fillers to enhance wear. Resin-based composites are not suited to function as endodontic post materials, however resin impregnated fibre reinforced composites are frequently used for the fabrication of posts. These posts predominantly use E-glass fibres, which are a mixture of amorphous phases with oxides of Si, Ca, Ba, Al and some alkali metal oxides.

Since it was evident from the results of the LDPE-HA-ZrO_2 study that a thermoplastic based composite may not fully satisfy the mechanical requirements for a post because of its lower stiffness, a subsequent Kevlar fibre reinforced Bis-GMA/TEGDMA/PMMA semi-IPN matrix with titanium dioxide nanofiller composite was designed as an alternative to fibre posts in current clinical use. In semi-IPN polymer, the linear phase and the cross-linked polymer phase are not bonded chemically together as is the case for a typical copolymer (Sperling 1994). This structure has previously been applied in fabrication of one commercial glass fibre IPN post (everStick® Post), which was found to have a better adhesion over the conventional cross-linked posts due to the ability of the bonding resin to diffuse into the linear phase of semi-IPN matrix (Lastumäki *et al.* 2003; Mannocci *et al.* 2008). However, the unfavourable coupling between the glass fibres and the semi-IPN matrix compromises the overall strength of everStick® post and a higher failure rate was observed clinically in teeth restored using glass fibre IPN posts (Cagidiaco *et al.* 2008a).

In general, the integrity of FRC depends on the choice of proper matrix and fibre material combination. The incompatibility between the components may result in continuous degradation in physical and mechanical properties of FRC posts under cyclic loading that simulates the normal occlusal and masticatory function (Stewardson *et al.* 2010; Barbizam and White 2014). Clinical studies have generally showed that debonding occurs during the restoration's lifetime with no noticeable tendency for immediate failure (Rasmick *et al.* 2010). Furthermore, the contact with oral fluids may produce flexion of fibre posts (Torbjörner *et al.* 1996; Mannocci *et al.* 2001; Drummond and Bapna 2003), with the consequent risk of secondary caries and debonding of the composite restorations of ETT (Soares *et al.* 2012). This is so because some FRC that are also used for fibre posts construction, especially glass and silica fibres, may be hydrolytically unstable (Vallittu 2014). Moisture absorption of carbon (Grant and Bradley 1995) and glass (Lassila *et al.* 2004) fibre-reinforced materials decreases the mechanical strength because of the degradation of the components. Therefore, a high-performance fibre with unique physicochemical properties, Kevlar 49® yarn, was selected to reinforce the semi-IPN in an attempt to overcome these shortcomings.

The inertness of the Kevlar fibre surface leads to ineffective interfacial adhesion, hence surface treatment using a combination of acetic acid etching and A-174 silanation pre-treatments was conducted that resulted in significantly increased flexural strength compared with untreated fibres. TiO_2 nanofillers were incorporated to offset the yellow colour of Kevlar, which is one of the ideal additives used to enhance the performance of polymeric materials because of the non-toxic nature and chemical stability (Zhang and Gao 2001). The content of the filler was selected on the basis of mechanical properties, radiopacity and aesthetics, and Fig. 6-1 shows the dependence of flexural properties on the filler content. The flexural modulus (FM) and strength (FS) improved with increasing particle weight fraction and at 25 wt.% increased FM and FS by nearly 14% and endowed the post with a white-coloured appearance and desirable radiopacity.

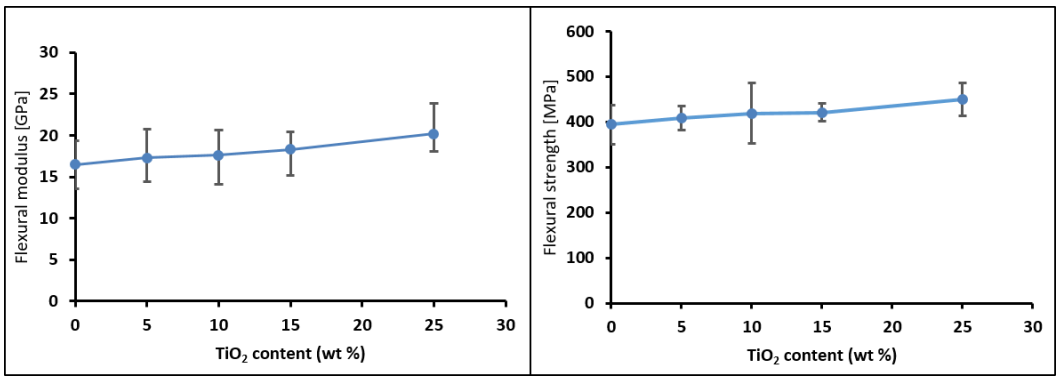

Figure 6-1 Flexural modulus (FM) and flexural strength (FS) of Kevlar fibre reinforced composites vs. TiO_2 nanoparticle content

In addition to appropriate flexural modulus (18.9 ± 1.6 GPa), which was close to that of dentine, and sufficient flexural strength (450 ± 35.9 MPa), the final formation of K FRC post material demonstrated higher thermal and cyclic fatigue resistance when compared with everStick® post. The high performance of K FRC could be attributed to the compatible combination of treated KF and semi-IPN based composite that enhanced the fibre-matrix interface, where failures more often occur. This is the first study to verify the suitability of the combination of these materials for the fabrication of root canal posts, which revealed sufficient ability to resist fatigue stress, thus suggesting considerable reliability of these materials when used clinically.

Beside good performance, biocompatibility is the most important characteristic of all dental materials. The cytocompatibility of PE-HA composites, which consist of well-known biocompatible materials, has been reported earlier in several studies (Di Silvio *et al.* 2002; Zhang *et al.* 2007; Alhashimi *et al.* 2013). Furthermore, similar composites have been successfully developed as bone analogue materials with excellent cytocompatibility (Bonfield *et al.* 1981; Deb *et al.* 1996). For K FRC, the results of MTT *in vitro* study demonstrated that KF posts possessed an acceptable level of cytotoxicity with minimum release of leachable toxic components. The biocompatibility of the components used in composite fabrication could explain these results. Methacrylate-based resins have been widely used in different dental materials. On the other hand, Kevlar fibre and fibre-PMMA composites have been previously identified as biocompatible materials (Henderson *et al.* 1987).

The second experimental section of the thesis focused on imparting an effective antibacterial property into post endodontic restoration to improve quality and durability for long-term success of ETT. Dental restorative materials such as polymeric adhesives or composite materials that exhibit antibacterial activity are useful for eliminating the harmful effects caused either by residual bacteria or bacterial microleakage (Siqueira 2002), that may result in recurrent caries and failure of both the restoration and the root canal treatment. The modification with inclusion of an antimicrobial agent that performs via contact killing rather than release of agents is advantageous in the oral environment. Researchers have focused on developing polymerisable compounds with strong antimicrobial activity upon contact that do not diminish over time nor affect the biocompatibility of the materials. To increase the antibacterial potency, the agent might need to be incorporated at high concentrations, which could significantly affect the overall polymer network structures and properties as associated with quaternary ammonium methacrylates (Imazato *et al.* 1999; Antonucci *et al.* 2012).

In this work, a potent polymerisable antibacterial monomer (EgMA) derived by functionalisation of eugenol, which is a well-known antimicrobial natural compound, was selected. Eugenol has been shown to be effective in reducing bacterial growth in a wide range of applications with a long history of success in clinical dentistry; eugenol-containing sealers remain the primary choice of endodontists, which has been proved to be the most effective against a range of microorganisms, including facultative anaerobes commonly isolated from infected root canals (Kaplan *et al.* 1999). Previously, Rojo *et al.* demonstrated that EgMA exhibits an antibacterial activity against both Gram-negative and Gram-positive bacteria (Rojo *et al.* 2006; Rojo *et al.* 2008), indicating that the eugenyl residue in the monomer maintains the bactericidal effect. This effect based on the presence of both 4-allylic and 2-methoxy groups contributes to the aseptic property of eugenol (Yuwono *et al.* 2002).

The monomer was successfully incorporated at 5 or 10 wt.% to prepare intrinsically antibacterial resin composites and further at 20 wt.% to modify two commercial adhesive systems without influencing their degree of conversion, curing properties and glass transition temperature (*T*g). The experimental composites were designed as dual-cured resin system to be applied for intracanal post cementation and core build-up in one-stage restoration whereas core build-up would immediately follow post cementation using the same composite material. Such a procedure could reduce the technique sensitivity, hazards of possible incompatibility of different composites, the working time of clinicians, and chairside time for patients (Sterzenbach *et al.* 2012). Interestingly, increasing EgMA content resulted in pronounced shear-thinning behaviour of the composites with excellent handling properties, low viscosity, and superior injectability into the root canal. All the mechanical properties of the composites were in the range accepted for clinical applications and comparable to that of Clearfil™DC Core, a commercial reference.

Highly established biocompatible fillers HA and ZrO_2 were used in composite formulations with weight fractions selected on the basis of optimal flexural properties and radiographic appearance. The bending test results showed that increasing the filler phase above 65 wt.% resulted in significant reduction in flexural strength (Fig. 6-2). The effect of EgMA, which allows for both crosslinking and forming branched structures, reduced the stiffness of the composites and increased the flexural and compression strength, as confirmed by both static and dynamic mechanical tests.

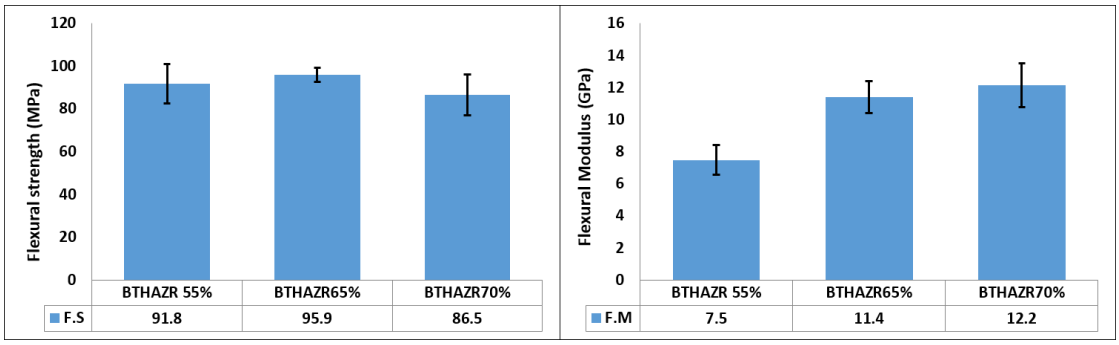

Figure 6-2 Optimisation of filler phase comprising hydroxyapatite (HA) and zirconium oxide (ZR) (4:3, wt./wt.) within BisGMA /TEGDMA, (B/T) resin based formulations

A high monomer concentration (20 wt.%) was added into adhesive resin formulations with the aim of imparting strong antibacterial effects. The addition was based on the results of a pilot study that showed no adverse effects on degree of monomer conversion and *T*g of Bis-GMA/HEMA (70/30 wt.%) blend polymers containing 20 wt.% EgMA (Table 6-1 and Fig. 6-3).

Table 6-1 Formulation and curing property (DC) of experimental bonding agents (n=3)

Group	Bond formulation	DC (%) 10 min after curing	DC (%) 24 h storage at 37 °C
BHEg0	**70 wt.% bis-GMA 30 wt.% HEMA CQ / EDAB (0.5wt./0.5wt.)***	**74 (0.5)**	**76 (1.0)**
BHEg10	**63 wt.% bis-GMA 27 wt.% HEMA 10 wt.% EgMA CQ / EDAB (0.5wt./0.5wt.)***	**70 (1.0)**	**74 (1.0)**
BHEg20	**56 wt.% bis-GMA 24 wt.% HEMA 20 wt.% EgMA CQ / EDAB (0.5wt./0.5wt.)***	**67 (1.3)**	**72 (1.3)**

*** The initiators 0.5% camphorquinone (QC) and activator 0.5% = 4-(dimethylamino) benzoate (EDAB), 1:1 molar ratio were added as wt.% with respect to monomer of the final resin monomers blend (100 wt.%) formulation.**

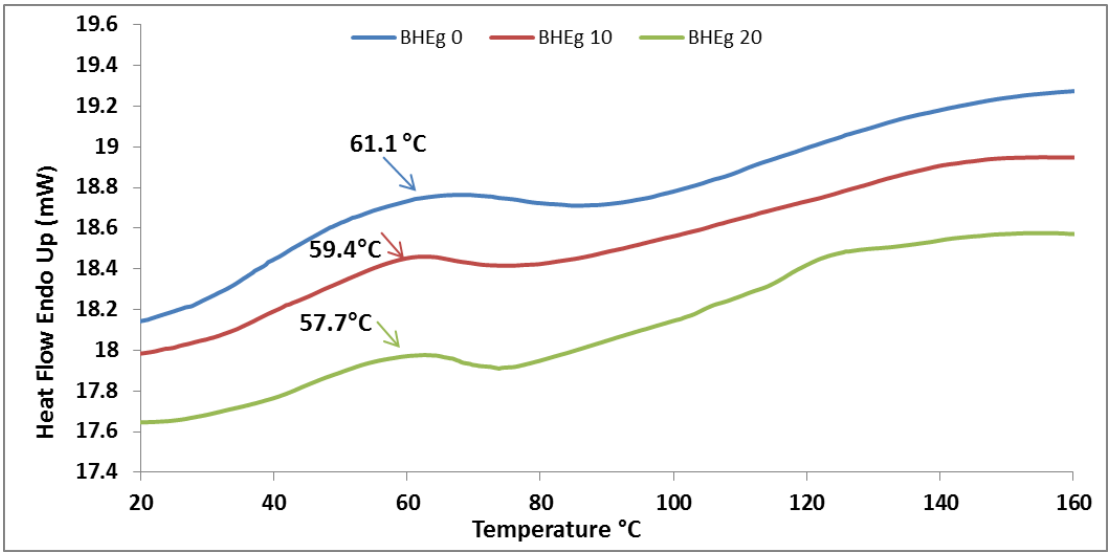

Figure 6-3 DSC representative curves of experimental bonding agents

The low viscosity of the EgMA monomer functioned as an excellent diluent for Bis-GMA in both resin composites and adhesives formulations. In the composites, it enhanced filler dispersion and resulted in a homogenous structure as shown in SEM images (Fig. 4.1-6), while in modified adhesives the lower viscosity improved the infiltration of the resins into the root dentine, especially with Modified Clearfil Universal Bond, that exhibited significantly greater bond strength in the middle region of the roots.

It can be assumed that the lower viscosity and modulus of polymeric composite containing EgMA will potentially reduce the polymerisation shrinkage stress within the material during early setting via resin flow and chain viscoelastic relaxation (Davidson and De Gee 1984; Labella *et al.* 1999), which can reduce the possibility of gap formation (da Silva *et al.* 2007), and enhance the bonding ability of the restorations. However, the important point established is that the addition of EgMA had no adverse effects on bondability to root canal dentine, evaluated by push-out test and SEM. According to the confocal observation of interfaces, composites containing EgMA showed an optimal integration to the adhesive layer with no air bubbles or gaps; the morphological features of resin-dentine interfaces created using EgMA modified adhesives were similar to their corresponding controls (unmodified adhesives) at 24 h and 6 M evaluation.

The antibacterial effectiveness of EgMA modified adhesives was initially evaluated by ADT before curing, which confirms the sensitivity of the three tested bacteria, *E. faecalis, S. mutans and P. acnes,* to uncured adhesives. From this point of view, incorporation of EgMA into adhesive resin is considered to be clinically important in providing a pre-cured antibacterial effect as cavity disinfectant agents against residual bacteria, while reducing operational chair time.

The antibacterial activity of cured materials was also studied in conjunction with their wettability. Composites containing EgMA act as a contact inhibitor against the bacteria which attach to the surface. The direct contact test performed against each single species showed the ability of the composite to inhibit bacterial growth, reducing the

number of colonies significantly. Further evaluation was made by growing a multispecies biofilm from these bacteria on the surface of adhesive resin discs for 14 days. Consistent results were obtained from biofilm inhibition test, indicating that EgMA based materials render bioactive bacteriostatic surfaces that reduce microbial resistance and biofilm formation.

The presence of EgMA monomer reduced the surface free energy, raising significantly the hydrophobic character of the corresponding material surface, which could increase the total interaction energy between the cytoplasm membranes of bacteria and the substrate. The inhibitory capacity of the monomer depends on the degree of interaction with the cell functions. Similar to this observation, increasing the alkyl chain length of quaternary ammonium salts increased the hydrophobicity, which was reported to enhance the reaction with cytoplasmic membrane of cells (Zhou *et al.* 2014).

The modification with 20% eugenol derivative, EgMA endowed the cured adhesives with antibacterial properties due to the presence of eugenyl residues anchored to the polymeric backbone, which were also demonstrated to possess hydrolytic stability and minimum water solubility. The combined effects of these adhesives to disinfect the root canal before curing and inhibition of bacterial growth after being cured would be useful to improve the success of endodontic restorations.

Cytotoxicity measurements indicated that all the polymeric systems were viable for *in vivo* applications. Cytocompatibility of the newly developed antibacterial materials is an absolute requirement that was confirmed via *in vitro* cellular studies using human gingival fibroblast cells. It is also important to highlight that the eugenol derivatives will be converted into high molecular weight copolymers after the curing reaction, leading to biocompatible cross-linked matrices. No deleterious effects were observed on cell viability and proliferation as shown by MTT and live/dead assay tests, suggesting the non-toxic nature in the oral environment and hence contributing to the clinical success of these composites as new antibacterial restorative materials.

6.2 Conclusions

The following conclusions can be drawn:

1. The suitability of thermoplastic polyethylene silanated hydroxyapatite with ZrO_2 composites designed to function as intracanal post material was limited, because of their lower rigidity, especially when small diameter posts are concerned. One particularly important finding is that the lower melting temperature of these composites would enable easy retrievability and further investigations are warranted.

2. Promising new fibre reinforced composite based on pre-treated Kevlar fibres and compatible semi-IPN were designed for restoration of ETT. The K FRC developed in this study was found to be suitable as an intracanal post material exhibiting superior mechanical stability, and fatigue resistance in comparison to everStick®POST, a commercial glass fibre/semi-IPN matrix post material in current clinical use. Furthermore, the material properties are expected to improve the durability of the endodontic restorations, with the additional advantages being favourable radiopacity and excellent biocompatibility.

3. The current study established that the incorporation of EgMA monomer into the resin composite and adhesive materials constitutes a novel approach to impart the antibacterial properties of eugenol to endodontic post restorations. This represents a significant improvement, considering that such materials were so far considered incompatible with *in situ* polymerising dental resin systems.

4. The specifically-designed dual-cure resin composites of 5 and 10 wt.% EgMA monomer and Bis-GMA/TEGDM resin system with 65 wt.% filler mixtures of HA/ ZrO_2 had suitable properties to find application for intracanal post cementation and core build-up restoration of ETT. The composite demonstrated proper initial handling viscosity facilitating their injection into the root canal, and provided an improvement in polymerisation exotherm, mechanical strength, viscoelasticity, radiopacity, and bonding

ability with results comparable to those of Clearfil™DC Core-Plus, a currently available commercial reference. Based on wettability and antibacterial tests, the immobilised EgMA monomer enhanced the hydrophobic factor of composite surfaces which exhibit effective bacteriostatic activity by reducing the number of CFUs of *E. faecalis, S. mutans and P. acnes* tested bacteria, commonly associated with failure of coronal and endodontic restorations.

5. EgMA modified adhesives showed an apparent cavity disinfecting effect before curing, and a significant inhibition of bacterial colonisation and biofilm formation on their surfaces after being cured, without any adverse effects on their degree of monomer conversion and bonding ability to root canal dentine.

6. Based on the cellular response, the experimental resin composites and modified adhesives containing EgMA prepared in the present study were found to be biocompatible and the results showed a high percentage of cell viability, suggesting the absence of any leaching monomer and preventing the potential toxic effect on surrounding tissues.

7. Finally, the combined use of K FRC post and EgMA containing resin composite and adhesive materials as a newly developed post-core system for the restoration of structurally compromised ETT would potentially improve the success of the restoration and the survival of endodontically treated teeth.

6.3 Suggestions for future work

Further improvement in mechanical strength of PE-HA composites fabricated in the current work can be accomplished by modulating the density of the polyethylene matrix or by self-reinforcing the matrix with UHMWPE fibres; thereby the generated structure possesses a higher stiffness and strength than the matrix and is well “bonded” to the matrix polymer.

The present study provides evidence that Kevlar FRC fabricated with semi-IPN matrix has potential as intracanal post material. Future work must involve the use of an automated method of post fabrication with a uniform distribution of K fibres. Further research is also needed to evaluate and improve the inter-diffusion bonding mechanism of suitable cements/composites resin monomer into the PMMA linear phase of the post matrix by modifying the solubility parameters.

The possible matrix metalloproteinase (MMP) inhibiting properties of EgMA containing materials developed in the present study need to be assessed; this will be a major advancement improving bonding stability of bonded restorations in this clinical procedure.

An effective post-core system could be established from the most suited optimised materials (post, luting resin composite core and adhesives) developed in the present research, which has paved the way for further studies to evaluate the performance and feasibility of this system to restore the structurally compromised ETT (*in vitro*). Nanoleakage, fracture resistance, and stress distribution analysis tests of post/tooth complex might be considered. Once the biomechanical properties and suitability of the system are fully assessed, a clinical assessment in a randomised clinical trial will be undertaken, evaluating the clinical significance of using these materials on long-term survival of ETT.

7. Appendices

7.1 Ethical approval for human teeth collection

NHS
Health Research Authority

NRES Committee London - Riverside
Bristol Research Ethics Committee Centre
Level 3 Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT

Telephone: 0117 342 1385
Facsimile: 0117 342 0445

17 January 2014

Reader Sanjukta Deb
Reader of Biomaterials,Biomimetics&Biophotonics
King's College London,Dental Institute
Biomaterials Research Group
Floor 17 Guy's Tower
King's College London,Dental Institute
SE1 9RT

Dear Dr Deb

Study title:	**Polyolefin composite fibres post material for the restoration of endodontically treated teeth**
REC reference:	**14/LO/0123**
Protocol number:	**NA**
IRAS project ID:	**144170**

The Proportionate Review Sub-committee of the NRES Committee London - Riverside reviewed the above application on 20 January 2014.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Miss Tina Cavaliere, nrescommittee.london-riverside@nhs.net

Ethical opinion

There were no ethical issues.

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

7.2 Materials manufacturers, description and their area of usage among research studies

Material	Manufacturer and description	Area of usage			
		PE-HA Composites (Chapter 2)	Kevlar FRC (Chapter 3)	EgMA containing resin composites (Chapter 4)	EgMA modified adhesive systems (Chapter 5)
Low density polyethylene (LDPE)	Goodfellow, Cambridge Limited England, powder, particle size: 1000 µm, Mw: 400,000 g/mol., density: 0.918 g/cm³, melting point: 140 °C.	X			
Hydroxyapatite (HA)	Plasma Biotal Ltd. (Derbyshire, UK), powder, particle size: 3-5 µm	X		X	
Zirconium oxide (ZrO_2)	Fisher Scientific Ltd., Loughborough, UK, powder, particle size:18 µm	X		X	
A-174 Silane coupling agent	Merck, Frankfurt, Germany, (3 Trimethoxysilyl propylmethacrylate)	X	X	X	
Acetic acid	BDH, VWR, Dorset, UK, concentration: 99.9%		X		
Kevlar 49®	Goodfellow Cambridge Limited, England. Yarn, 12 µm filament diameter, density: 1.44 g/cm^3		X		
PMMA	Aldrich, UK, particles, Mw~350000 g/mol.		X		
Titanium dioxide (TiO_2)	Aldrich, UK, nanopowder, particle size: <100 nm		X		
Bis-GMA	Esschem Europe Ltd Ltd (Durham, UK), monomer, Mw: 512.5 g/mol., density: 1.161 g/mL at 25 °C		X	X	
TEGDMA	Esschem Europe Ltd Ltd (Durham, UK), monomer, Mw: 286.3 g/mol., density: 1.092 g/mL at 25 °C		X	X	
Benzoyl peroxide (BPO)	Merck, Frankfurt, Germany, heat initiator Purified by fractional crystallisation from ethanol		X	X	
Camphoroquinone (CQ)	Sigma-Aldrich, Company Ltd, Dorset, UK, Light initiator			X	X
N, N-dimethyl-p-toluidine (DMpT)	Sigma-Aldrich, Company Ltd, Dorset, UK, (activator)			X	
Eugenol	Sigma-Aldrich, Company Ltd, Dorset, UK			X	X
Methacryloyl chloride (95%)	Alfa Aesar, UK			X	X
Trimethylamine	Sigma-Aldrich, Company Ltd, Dorset, UK			X	X
Eugenyl Methacrylate (EgMA)	Synthesised monomer. Mw: 232.23 g/mol.			X	X
Ethyl-4-dimethyl-aminobenzoate (EDAB)	Sigma-Aldrich, Company Ltd, Dorset, UK, (activator)				X

7.3 The commercial materials used in research studies

Material	Manufacturer	Description	Patch number	Study
RelyX™ Fibre Post	3M ESPE, USA	Prefabricated, glass fibres in epoxy resin matrix. Fibre content: 75 vol.%		PE-HA Composites (Chapter 2)
Everstick® Fibre Post	Stick Tech Ltd Turku, Finland	Individually formed, silanised pre-impregnated unidirectional glass fibres in Bis-GMA/PMMA semi-IPN matrix. Fibre content: 60-65 vol.%	2050426-ES-125	Kevlar FRC (Chapter 3)
Clearfil™DC Core plus	Kuraray, Tokyo, Japan	Dual –cured, two pastes system. Composition: Bis-GMA, TEGDMA, aliphatic methacrylates, aromatic methacrylate, silanated colloidal silica, colloidal silica, barium glass, aluminium oxide, dl-Camphorquinone initiators, accelerators and pigments. Fillers content: 74 wt.% (52 vol.%). Particle size range: from 0.01 to 20 µm	A90137	EgMA containing resin composites (Chapter 4)
Clearfil Universal Bond™	Kuraray, Tokyo, Japan	Self-etch, single bottle component MDP, Bis-GMA, HEMA, hydrophilic aliphatic dimethacrylate, colloidal silica, silane coupling agent, CQ, ethanol, water	1562R041R	EgMA modified adhesive systems (Chapter 5)
Adper Scotchbond™ multi-purpose plus	3M ESPE, St Paul, MN, USA	Total-etch, multiple components Etchant: 35% Phosphoric acid gel Activator: Ethyl alcohol, sodium benzenesulfinate Primer: Water, HEMA, copolymer of acrylic, itaconic acids Adhesive: Bis-GMA, HEMA, Tertiary amines, photiinitiator Catalyst: Bis-GMA, HEMA, Benzoyl peroxide	N662538	EgMA modified adhesive systems (Chapter 5)

7.4 List of publications in international peer-reviewed journals

1. ALMAROOF, A., ALHASHIMI, R., MANNOCCI, F. & DEB, S. 2015, New functional and aesthetic composite materials used as an alternative to traditional post materials for the restoration of endodontically treated teeth. Journal of Dentistry, 43, 1308-1315.

2. ALMAROOF, A., ROJO, L., MANNOCCI, F. & DEB, S. 2016, A resin composite material containing an eugenol derivative for intracanal post cementation and core build-up restoration. Dental Materials, 32, 149-160.

3. ALMAROOF, A., NIAZI, S. A., ROJO, L., MANNOCCI, F. & DEB, S. 2016, Influence of a polymerizable eugenol derivative on the antibacterial activity and wettability of a resin composite for intracanal post cementation and core build-up restoration. Dental Materials, 32, 929-939.

4. ALMAROOF, A., NIAZI, S. A., ROJO, L., MANNOCCI, F. & DEB, S. 2017, Evaluation of dental adhesive systems incorporating an antibacterial monomer eugenyl methacrylate (EgMA) for endodontic restorations. Dental Materials, doi: 10.1016/j.dental.2017.01.016 (in press).

5. A. Almaroof, F. Mannocci, S. Deb. 2015. Biomechanical composites for restoration of root filled teeth, (conference paper). International Endodontic Journal, 0 (Suppl. 0), 1–90. European Society of Endodontology ESE Biennial Congress, September 16-19, 2015, Barcelona, Spain

6. Ahmed. Almaroof, Luis Rojo, Francesco Mannocci, Sadia Niazi & Sanjukta Deb 2016, Intrinsically antibacterial adhesive systems based on polymeric derivative of eugenol, (conference paper). Dental Materials, Volume 32, Supplement 1, Pages e2–e3. The Academy of Dental Materials (ADM) Annual Meeting, October 12-15, 2016, Chicago, Illinois, USA.

7. ALMAROOF, MANNOCCI, F. & DEB, S. 2017, New semi-interpenetrating network composites reinforced with Kevlar as intracanal post material for restoration of endodontically treated teeth, (submitted to Operative Dentistry, #: 17-096-L).

7.5 List of abstracts submitted to national and international conferences

1. Ahmed Almaroof, Sanjukta Deb, Francesco Mannocci, “Biomechanical composites for restoration of endodontically treated teeth” (oral presentation), 17th Annual Postgraduate Research Day at King’s College London Dental Institute (2015).

2. Ahmed Almaroof, Sanjukta Deb, Francesco Mannocci, “A novel resin composite material containing an eugenol derivative for intracanal post cementation and core build-up restoration” (oral Presentation),18th Annual Postgraduate Research Day at King’s College London Dental Institute (2016).

3. Ahmed Almaroof, Sanjukta Deb, Francesco Mannocci, “Development and Characterisation of Biomechanical Composite for Restoration of Endodontically Treated Teeth” (oral presentation), RSC: Biomaterials Chemistry Special Interest Group Annual Conference: 5-6 January (2015), London, UK.

4. Ahmed Almaroof, Luis Rojo, Francesco Mannocci, Sadia Niazi & Sanjukta Deb., “New eugenol derivative containing luting resin composite core materials for endodontic restorations” (poster presentation), ConsEuro 2015 Conference: 14th – 16th May (2015), QEII Centre in London, UK.

5. A. Almaroof, F. Mannocci, S. Deb. “Biomechanical composites for restoration of endodontically treated teeth” (poster presentation), European Society of Endodontology, ESE Biennial Congress, Barcelona, Spain, 17th - 19th September (2015).

6. Ahmed Almaroof, Luis Rojo, Francesco Mannocci, Sadia Niazi & Sanjukta Deb (poster presentation) “Intrinsically antibacterial adhesive systems based on polymeric derivative of eugenol”. The Academy of Dental Materials (ADM), Chicago, Illinois, USA, October 12-15, 2016.

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